Badger social networks and their implications for disease transmission

Submitted by:

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

“A lendri,” said Bigwig. “I’ve heard about them in the Owsla. They’re not really dangerous. They can’t catch a rabbit that runs, and nearly always you can smell them coming”.

Richard Adams, Watership Down

Abstract

1. Diseases that infect wildlife populations pose a significant threat to public health, agriculture, and conservation efforts. The spread of these diseases can be influenced by the social structure of the population, and, therefore, often need to be accounted for in disease models.
2. In this thesis I use high-resolution contact data to explore the social structure of a high-density population of European badgers (*Meles meles*). I explore how this structure might influence the spread of bovine tuberculosis (bTB), a debilitating disease of cattle for which badgers are a wildlife reservoir. Denning and home range data collected using radio tracking is also used to determine how this social structure is related to badger space use.
3. I use social network analysis to identify the community structure of the badger population, revealing that badgers interact in fewer, more distinct groups than previously assumed. This is likely to inhibit the spread of disease through the population, given that the probability of infection entering a new social group will be reduced. However, among-group contact is still found to occur even between the most isolated groups.
4. I show that this among-group contact is more likely to occur between less related individuals, possibly suggesting that breeding behaviour may drive among-group contact as a mechanism for inbreeding avoidance.
5. To gain additional insight into this among-group contact, I determine how badger spatial behaviours are related. I show that the use of dens (setts) away from the social group's main sett (outlier setts) in the spring is associated with extra-territorial ranging. I also show that this extra-territorial ranging is associated with more central network positions. The seasonality of this behaviour further suggests that this may be related to breeding activity.
6. These findings suggest that behaviours associated with extra-group ranging may increase the risk of acquiring and transmitting infection. Therefore, use of outlier setts in the spring could act as a spatial proxy to identify high-risk individuals for disease spread, offering potential targets for disease control.
7. Finally, I discuss the implications of these findings in regard to what they reveal about badger behaviour, disease transmission, and the design of effective disease control strategies. The importance of understanding population social structure for the study of wildlife disease in general is also discussed.

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Author's declarations

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Chapter 2 - The raw contact data for this chapter were collected by Nicola Weber.

Chapter 3 - The raw contact data for this chapter were collected by Nicola Weber. Relatedness estimates were calculated by Clare Benton

Chapter 1
General Introduction

1.1 The implications of wildlife disease

Diseases that infect wildlife populations can pose a serious threat to public health (Jones *et al.* 2008a). Zoonotic diseases can be transmitted between humans and animals, and make up approximately 70% of human emerging infectious diseases (Taylor, Latham & Woolhouse 2001; Jones *et al.* 2008a). The consequences of these emerging diseases can be devastating, as recently illustrated by the 2013-2016 Ebola outbreak in West Africa, that resulted in 11,000 reported deaths (WHO Ebola Response Team 2016). In addition to a new source of diseases, wildlife can act as reservoirs of infection that maintain disease in the environment. For example, many mammal species can be infected with rabies, sustaining the disease and infecting humans through biting (Rupprecht, Hanlon & Hemachudha 2002). Wildlife reservoirs of disease can also infect domestic animal populations, creating a further public health risk. For example, human cases of brucellosis infection from wild populations of elk (*Cervus canadensis*) and bison (*Bison bison*) are relatively rare (Young 1995). However, brucellosis can be transmitted from these wild populations to domestic cattle (Cheville, McCullough & Paulson 1998), and therefore increase human exposure (Young 1995).

Wildlife disease can also have highly detrimental effects for livestock farming, for example the economic losses associated with cattle brucellosis can be extremely large, costing $600 million per annum in Latin America alone (Seleem, Boyle & Sriranganathan 2010). Before it was eradicated, rinderpest caused great damage to African agriculture, with 95% of domestic cattle populations lost in some regions (Roeder 2011). This rinderpest pandemic also led to the death of 95% of the wild ungulate population, changing much of the sub-Saharan ecosystem (Roeder 2011). This example illustrates the implications wildlife disease can also have for conservation. Endangered species are particularly vulnerable to disease, with canine distemper virus contributing to the decline of the African wild dog (*Lycaon pictus*) (Woodroffe & Ginsberg 1999), and devil facial tumour disease (DFTD) threatening the Tasmanian devil (*Sarcophilus harrisii*) with extinction (McCallum *et al.* 2009).

Through studying diseases and how they spread, the reasons why some infections remain local and endemic to a region, while others become a

widespread epidemic, can be understood. For example, studying HIV has revealed that the movement of people from rural areas to cities allowed disease to spread along regional transport links (Faria *et al.* 2014), until the migration of workers facilitated the international spread of the disease (Gilbert *et al.* 2007). This migration led to a global pandemic that has infected over 70 million people, with an estimated 36.7 million people living with HIV in 2015 (UNAIDS 2016). Information regarding the spread of disease can be used to plan effective disease control strategies. For example, showing how effective airport screening would have been in controlling the 2003 SARS outbreak, means that this information can be used to advise future disease management strategies (Bowen & Laroe 2006). Therefore, fully understanding how disease spreads through populations can increase the efficacy of disease control.

1.2 Epidemiology and disease models

Traditional epidemiological models

Mathematical disease models can be used to predict how an infection will spread through a population, allowing potential control strategies to be tested. This makes them a highly valuable tool for epidemiology. The SIR model determines how quickly a disease will spread through a population, by splitting individuals into separate states of 'Susceptible', 'Infected', or 'Recovered'. The average rate that susceptible individuals become infected is then calculated (Anderson, May & Anderson 1992). This transmission coefficient is a product of the number of susceptible individuals in the population, the rate of contact, the probability that contact is with an infected individual, and the probability of disease being transmitted given contact with an infected individual (Begon *et al.* 2002; Keeling & Rohani 2008).

The information from the SIR model can be used to calculate the reproductive rate (R_0) of a disease to determine if an epidemic is likely to occur. R_0 describes the average number of secondary cases that arise from an average primary case, in an entirely susceptible population (Anderson *et al.* 1992). Therefore, if an epidemic is to occur, R_0 must be greater than one. The reproductive rate can help predict the efficacy of different disease control strategies. For example, an epidemic can be stopped if R_0 can be reduced to below one (Anderson *et al.* 1992). This could be achieved by reducing the number of susceptible

individuals in the population using vaccination (Anderson *et al.* 1992). If R_0 is very high, then a large proportion of the population will need to be vaccinated, but herd immunity could be achieved with lower vaccination levels if R_0 is very low. R_0 has successfully been used in modeling studies to show that current targets of dog vaccination levels are not high enough to effectively control the spread of rabies in Kenya (Kitala *et al.* 2002). Alternatively, R_0 can be reduced through lowering the number of both susceptible and infected individuals using culling (Anderson *et al.* 1992). For example, modeling studies have shown that by quickly culling all cattle on farms with foot and mouth disease, R_0 can be reduced and the disease more effectively controlled (Ferguson, Donnelly & Anderson 2001).

When individuals within the SIR model are given the same transmission coefficient (beta) based on the population average (Anderson *et al.* 1992; Begon *et al.* 2002; Keeling & Rohani 2008; Beldomenico & Begon 2010), it is effectively assumed that all individuals in the population have the same susceptibility to infection, infectiousness and contact rates (Beldomenico & Begon 2010). However, in reality, individual variation will affect disease spread, with individuals varying in their susceptibility to infection and their contact rates (Beldomenico & Begon 2010). For example, Anderson and May (1987) calculated the R_0 of a sexually transmitted disease based on the population average, assuming that all individuals have the same number of sexual contacts (May & Anderson 1987). However in reality, some individuals will have more contacts than others, resulting in the transmission coefficient for individuals with very few sexual contacts being overestimated, and those with very many underestimated (May & Anderson 1987). Not acknowledging individual variation in these models can, therefore, affect the accuracy of the estimates produced.

To determine how much variation in contact rates can influence the accuracy of these models, models that assume homogenous contact have been compared to those that assume heterogeneous contact (Keeling 2005; Lloyd-Smith *et al.* 2005; Bansal, Grenfell & Meyers 2007). The SIR model was found to largely agree with estimates from models that accounted for contact rate variation, particularly when the contact structure was reasonably homogenous (Bansal *et*

al. 2007). However, differences between model estimates increased when contact rates became more heterogeneous, with the SIR model underestimating the initial speed of disease spread, and therefore underestimating R_0 (Keeling & Eames 2005; Bansal *et al.* 2007; Grassly & Fraser 2008). This meant that the proportion of the population requiring control in order to stop an epidemic was also underestimated, and the efficacy of the control that was deployed overestimated (Keeling & Eames 2005; Bansal *et al.* 2007; Grassly & Fraser 2008). In addition, differences between model estimates were exaggerated further in populations with community structure, where individuals live in sub-groups within the population (Jones & Salathe 2010). Therefore, in populations with heterogeneous contact structures, it is clear that disease transmission cannot be fully understood if contact rate variation is ignored (Lloyd-Smith *et al.* 2005). Social networks have the potential to further the understanding of disease transmission in this direction (Newman 2002).

Network theory

Networks have been extensively studied in a range of disciplines including mathematics, physics, sociology, psychology and biology (Oliveira & Gama 2012). The ability to use networks to conceptualise complex social structures in a simple way makes them of particular value to epidemiology. Networks consist of a group of entities and the relationships between them, for example individuals in a population and their interactions. These networks can be built from relational data, and visualised in the form of a network graph where nodes represent individuals and edges represent the relationships that occur between them (Figure 1.1a) (Oliveira & Gama 2012). These edges can be directed to indicate the direction of the interaction, and weighted to represent the strength of the interaction. For example, edges could be binary to represent the presence or absence of an interaction, or weighted to represent the frequency or duration of an interaction (Figure 1.1b). This allows rare interactions to be distinguished from common ones. Edges can identify pathways for transmission, or potential transmission, of infections through a population. Therefore, networks can be used to help understand and predict how a disease might spread, and determine to what extent this is a product of the social structure of the population (Newman 2002; Danon *et al.* 2011). This information can then be used to design disease control strategies (Danon *et al.* 2011).

To identify the individuals and relationships that are most important for disease transmission, networks can be analysed using social network analysis (SNA). Theoretical networks based on simulations have been extensively explored to give insight into how different network structures can alter the spread of disease (for example: Keeling 2005; Martínez-López, Perez & Sánchez-Vizcaíno 2009; Danon *et al.* 2011). For empirical networks, SNA can also provide metrics to highlight specific aspects of the social structure that may be important for disease transmission. For example, the network properties of nodes can identify which individuals are likely to be important for disease transmission. These metrics include degree centrality, which is a measure of how much direct contact an individual has with others in the network (Figure 1.1c) (Hawe, Webster & Shiell 2004). Closeness centrality reflects how connected an individual is to all others in the network. Unlike degree, closeness also includes indirect contacts, and reflects the number of steps it takes to reach all others in the network (Hawe *et al.* 2004). Flow-betweenness is a measure of how important an individual is as a point of social connection, based on the number of times an individual connects nodes that otherwise would not have been able to reach each other (Hawe *et al.* 2004). Individuals with high flow-betweenness often act as bridges between different communities. These communities emerge when edge density varies in different regions of the network, leading to sub-groups forming where there is a high density of contacts within groups, but a low density of contacts among groups (Figure 1.1d) (Newman 2002; Oliveira & Gama 2012). Community structures like this are a common feature of networks that can also be identified using SNA.

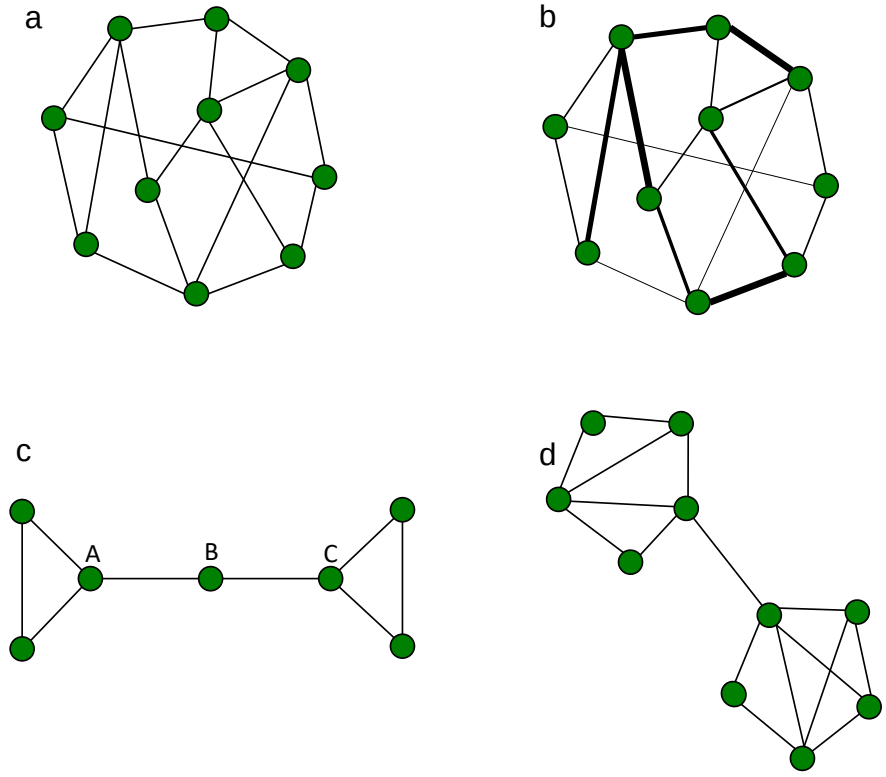


Figure 1.1 Examples of different network characteristics (a) A simple undirected 10-node binary network with no community structure. (b) The same network with weighted edges. (c) A network where nodes A and C have a degree centrality of 3, and all other nodes have a degree centrality of 2. Node B has the highest closeness centrality with the furthest node 2 steps away, compared to node A where the furthest node is 3 steps away. Node B also has the highest flow-betweenness centrality, indirectly connecting nodes A and C. Note that B has the highest flow-betweenness, but not the highest degree. (d) A simple 10-node network with two distinct communities. Networks are recreated from Wey *et al.* 2008.

Disease spread in social networks

Theoretical networks have been extensively explored to give insight into how network structure can alter the spread of disease. For example, in a random network each node has a fixed number of randomly connected contacts (Figure 1.2a). In this type of network, disease spreads through a branching process, with transmission slowing over time as the number of susceptible hosts diminishes (Keeling & Eames 2005). However, given that social contacts are seldom random, this type of network is rarely found empirically in wild

populations. In comparison, lattice networks are highly structured with each node connected to all adjacent nodes (Figure 1.2b). This results in the network being highly clustered locally, but globally having very long path lengths between a focal node and all others in the network. Therefore, disease spreads slowly in waves across the population (Keeling & Eames 2005). However, like random networks, lattice structures are not reflective of real life networks given that population connectivity is rarely exclusively local and often has long-ranging contacts (Danon *et al.* 2011).

Small world networks are a hybrid of these two network types, resembling a lattice but with the addition of a few, random long-ranging contacts (Figure 1.2c). This structure results in high levels of both local and global connectivity, allowing disease to spread quickly through the population (Keeling & Eames 2005). However, compared to random networks, overall outbreak size can be smaller due to saturation of the local network leading to the local extinction of infection (Christley *et al.* 2005). This may suggest that disease models that assume random mixing overestimate overall outbreak size (Christley *et al.* 2005). Networks with small world properties have been observed in lion (*Panthera leo*) populations in the Serengeti. Although lions live in separate prides, creating a highly clustered local network, rare contact between these prides are sufficient enough to make these networks small world (Craft *et al.* 2011). Networks like this can facilitate the transmission of infection across the whole population.

Scale-free networks consist of some individuals that have many connections and others very few. These networks can be created through adding individuals to a network one by one, preferentially connecting each new individual to those already with many connections (Keeling & Eames 2005). Therefore, scale-free networks often contain superspreaders; individuals that are disproportionately responsible for disease spread. For example, extreme heterogeneity in contact rates in deer mice (*Peromyscus maniculatus*) means that only 20% of the population is responsible for potentially 80% of the transmission of Sin Nombre virus (SNV) (Clay *et al.* 2009).

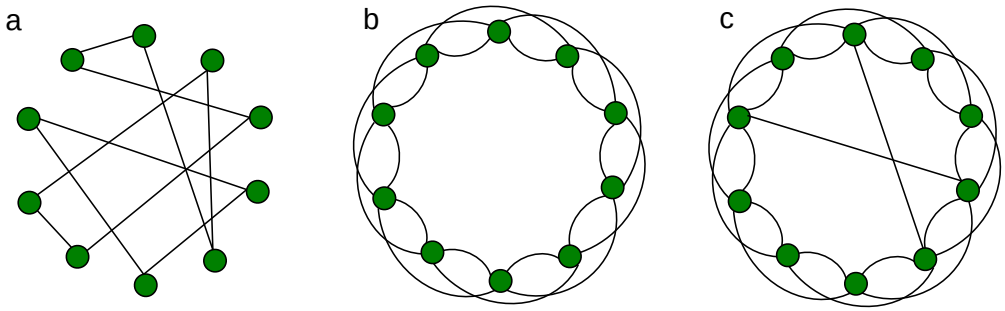


Figure 1.2 Three different types of theorised network. (a) A random network, with each node having a degree centrality of 2. (b) A lattice network with each node having a degree centrality of 4. This network is highly clustered locally, but globally has very long path lengths. (c) A small world network that is both locally clustered and globally connected. Networks recreated from Keeling & Eames 2005.

At the individual level, how connected an individual is will influence their risk of acquiring infection, with individuals that are more central in the network having a higher risk than those that are more isolated (Christley *et al.* 2005). For example, gidgee skinks (*Egernia stokesii*) that are infected with ticks and blood parasites have higher degree scores, suggesting that higher contact rates increases susceptibility to infection (Godfrey *et al.* 2009). Similar patterns have been found in sleepy lizards (*Tiliqua rugosa*) where individuals with higher weighted degree scores are more likely to be infected with *Salmonella* (Bull, Godfrey & Gordon 2012), and in brushtail possums (*Trichosurus vulpecula*) where individuals with higher closeness and flow-betweenness scores are more likely to acquire bovine tuberculosis (Corner, Pfeiffer & Morris 2003).

The community structure of a network can also influence disease spread. Within a population, communities can arise when individuals preferentially associate with others. This clustering commonly occurs between individuals that are similar, for example those that are of a similar age (Wey & Blumstein 2010), personality type (Massen & Koski 2014) or related (Widdig *et al.* 2001). The topology of these communities can have implications for disease spread. For example, Tasmanian devil populations exhibit little community structure, with all individuals effectively belonging to a single group (Hamede *et al.* 2009). This means all individuals are at risk of contracting DFTD should it enter the

population, posing a severe conservation risk to this already endangered mammal. However in populations with strong community structure, overall infection risk can be reduced (Wu & Liu 2008). Although individuals are more likely to contract infection from members of their own community, they are less likely to contract infection from individuals in other communities (Jones & Salathe 2010). This can lead to local outbreaks dying out before infection can reach another group, reducing the likelihood of a wider epidemic occurring (Jones & Salathe 2010). The protective nature of community structure has been empirically observed in primate populations, where strong community structure is associated with reduced parasite load (Griffin & Nunn 2012).

Individuals that bridge sub-groups and dilute the community structure of a population can therefore be highly influential for disease spread. These important bridging behaviours can often be related to space use. For example, larger range sizes observed in black-backed and side-striped jackals (*Canis mesomelas* and *Canis adustus*) during the mating season led to elevated contact rates among groups, and an increase in rabies transmission (Loveridge & Macdonald 2001). Home range overlap can also influence contact rates, as seen in male urban red foxes (*Vulpes vulpes*) that frequently enter neighbouring territories to seek extra-group mating events (White & Harris 1994). However, the influence that these bridging behaviours have on disease spread depends on the infection in question. For example, in the Serengeti lion population, nomads that connect social groups were found to only increase transmission of diseases with long infectious periods, such as feline calicivirus, due to the time needed to travel between prides (Craft *et al.* 2011).

Social networks and disease control

In order to control disease spread, vaccination can reduce the number of susceptible individuals in a population (Anderson *et al.* 1992). Vaccination both increases the number of immune individuals, resulting in fewer infections, and reduces the probability that any unvaccinated individuals will encounter an infected individual (Anderson *et al.* 1992). Traditionally disease management has been achieved through blanket control policies. However social network analysis can identify individuals that are disproportionately important for disease spread, allowing individuals to be targeted for a more efficient disease control

strategy (Grassly & Fraser 2008; Jones & Salathe 2010). For example, the superspreading individuals that are often identified in scale-free networks offer a potential control target. Focussing half of disease control on the most infectious 20% of individuals has been shown to be three times more effective at controlling disease spread than random control (Lloyd-Smith *et al.* 2005). Such targeted control strategies are especially viable if these individuals share a distinguishing attribute. This was the case in the deer mice example, where older, heavier mice are likely to be disproportionately responsible for SNV transmission (Clay *et al.* 2009). If these individuals could be vaccinated before acquiring infection, then a large proportion of disease transmission events could be prevented.

Similar to superspreaders, central individuals in the network can also offer vaccination targets, to prevent them from acquiring infection and limiting the subsequent spread of disease (Christley *et al.* 2005). This strategy was found to be viable for chimpanzees (*Pan troglodytes schweinfurthii*), where simulations showed that selectively vaccinating chimps with high degree scores can reduce the overall number of individuals requiring vaccination by up to 35% (Rushmore *et al.* 2014).

Alternatively, decreasing population density can reduce contact rates. For example, culling brushtail possums by 60% prevents den sharing (Caley *et al.* 1998), which is known to increase the risk of disease transmission (Corner *et al.* 2003). However, care must be taken to ensure the removal of individuals does not cause the network to destabilise and fragment. This was seen in simulations of pigtail macaque (*Macaca nemestrina*) populations, where the removal of individuals led to an increase in aggression, and eventual fragmentation of the social structure (Flack, Krakauer & de Waal 2005). Perturbations such as these can be counterproductive for disease control.

In populations that have a strong community structure, targeting individuals that have high contact rates might not be the best strategy (Jones & Salathe 2010). Instead, targeting the individuals that bridge communities can be more effective than targeting those with high degree (Krause, Croft & James 2007; Jones & Salathe 2010). This is because individuals that have few contacts but bridge

communities will facilitate disease spread to areas of the network that might otherwise have remained free of infection. Consequently, they will likely be more important for disease spread compared to individuals that only have high within-group degree scores (Jones & Salathe 2010). Therefore, the design of disease control strategies can benefit from taking into account the community structure of the population.

Determining contact structures

When studying disease spread, only interactions that are relevant to transmission should be included in the network (Keeling & Eames 2005). Therefore, the type of contact data that is required will depend on the infection in question. For example, when studying a sexually transmitted disease only information regarding sexual contacts should be included in the network. For human diseases, these data can be obtained by asking individuals who they have had sexual contact with, a process otherwise known as contact tracing (Klovdahl *et al.* 1994). Defining contacts for the transmission of airborne infections can be less clear, but networks weighted by time or duration can identify lengthier contacts that may be more important for disease transmission (Keeling & Eames 2005). Behavioural studies can also help identify high-risk behaviours to allow relevant contacts to be defined. For example, meerkats (*Suricata suricatta*) that groom others have elevated risk of acquiring bovine tuberculosis (Drewe 2010), indicating that grooming interactions should be included in the network. Networks can also be built for diseases that are transmitted indirectly. For example, gidgee skinks can be infected with ticks that carry blood parasites, and so sequential use of the same rock crevice can provide opportunities for indirect transmission between individuals (Godfrey *et al.* 2009).

Once the type of contact that should be included in the network has been identified, the most appropriate method of collecting these data will depend on the species in question. For example, meerkats can be directly observed (Drewe 2010), but this technique is only suitable for populations habituated to human presence and is less appropriate for nocturnal and cryptic species (Ji, White & Clout 2005). However, capture-mark-recapture can be used to infer contact rates between individuals that are less easy to observe, such as

brushtail possums. This method infers contact rates based on overlapping home ranges (Porphyre, McKenzie & Stevenson 2011). In the context of disease transmission, this accounts for both direct contacts that may occur between possums, and indirect contacts that may occur through environmental contamination. This method is particularly accurate when organisms occur at high densities (Perkins *et al.* 2009). Radio tracking can also calculate spatial overlap to infer contact rates, and is generally regarded to be better at detecting rare contacts that occur in low density populations (Perkins *et al.* 2009). This method has been used with success to measure infrequent among-group contacts in urban red foxes (White & Harris 1994). However, this method is labour intensive (Cross *et al.* 2009), and data are only obtained for the period of time that tracking is carried out.

Automated proximity loggers provide continuous, remote collection of high-resolution data on direct interactions between tagged individuals (Marsh *et al.* 2011). Their long battery life and large memories means that they require little maintenance or intervention from the observer once they are deployed, making them especially appropriate for animals that range over large distances (Krause, James & Croft 2010). These loggers record when and for how long two tagged individuals interact, making them especially useful to detect potential transmission events for diseases that require close contact. For example, these loggers have been previously used with success in wild populations of brushtail possums (Ji *et al.* 2005), meerkats (Drewe 2010), and European badgers (*Meles meles*) (Böhm *et al.* 2008; Weber *et al.* 2013a; Drewe *et al.* 2013), to give insight into the disease dynamics of bovine tuberculosis.

1.3 Bovine tuberculosis

Bovine tuberculosis transmission and control

Bovine tuberculosis (bTB) is a chronic, debilitating disease of cattle, caused by the bacterium *Mycobacterium bovis.* This bacterium is highly related to the causative agent of human tuberculosis, *M. tuberculosis* (Garnier *et al.* 2003), and can infect both human and animal hosts. Therefore, *M. bovis* poses a global public health risk from human contact with infected cattle, contaminated meat products and unpasteurised milk (Bengis *et al.* 2004; Torgerson & Torgerson 2009; Cadmus *et al.* 2010).

Among cattle, *M. bovis* is predominantly transmitted through aerosol inhalation (Neill *et al.* 1994), with high stocking densities thought to increase disease transmission rates (Neill *et al.* 1989). However *M. bovis* can survive in the environment for several months (Young *et al.* 2005), resulting in any excretions from infected individuals posing an additional transmission risk. In order to control disease spread, bTB is largely managed in developed countries through extensive test-and-slaughter programmes and restrictions upon trade and animal movements (Krebs *et al.* 1997; Michel, Müller & van Helden 2009). This results in high levels of economic loss from reduced cattle productivity, loss of trade, and compensation pay-outs (Michel *et al.* 2009). However, the presence of wildlife reservoirs of disease can complicate the control of bTB. Generally, disease reservoirs emerge when cattle bTB infection spills over into wildlife populations. These reservoirs are then able to transmit disease back into cattle in a re-infecting cycle (Daszak, Cunningham & Hyatt 2000). Wildlife reservoirs of bTB include American bison, white-tailed deer (*Odocoileus virginianus*), African buffalo, wild boar (*Sus scrofa*), brushtail possums, and European badgers (Bengis *et al.* 2004).

Badgers and bovine tuberculosis

In the UK, badgers were first discovered to carry bTB infection in 1971, when a tuberculous badger was found on a farm in South West England (Muirhead, Gallagher & Birn 1974). Subsequent studies have shown that bTB infection in badgers is widespread across Britain (Cheeseman, Wilesmith & Stuart 1989). The pathogenesis of bTB infection in badgers can be complex; exposure to infection does not necessarily lead to the development of disease, with many badgers harbouring latent infections that do not progress any further, and in some cases can be completely resolved (Gallagher & Clifton-Hadley 2000). Other exposed badgers may develop lesions. If these lesions rupture then the badger may become infectious and the disease may progress, leading to poor body condition and eventual death (Gallagher & Clifton-Hadley 2000). However survival rates of exposed and infectious badgers can remain relatively high (Graham *et al.* 2013), with infected females able to successfully reproduce (Cheeseman *et al.* 1989).

The high prevalence of pulmonary lesions in tuberculous badgers suggests that bTB is predominantly transmitted through the respiratory route (Gallagher & Nelson 1979). Given that repeated exposure to TB test-positive individuals is necessary for successful TB transmission in other species (Porphyre *et al.* 2011), individuals that spend more time together are likely to be at greater risk of sharing infections. This is likely to be particularly true for individuals that rest together, given that badgers sleep in underground burrows (setts) where respiratory conditions are poor (Roper 1992). Pseudo-vertical transmission of TB in badgers may also be possible, with cubs more likely to become infected if highly related TB test-positive females are present (Tomlinson *et al.* 2013; Benton *et al.* 2016). In addition, *M. bovis* can be transmitted through bite wounding, which generally results in a more severe infection (Gallagher & Nelson 1979; Cheeseman *et al.* 1988; Jenkins, Cox & Delahay 2012). An increase in tuberculous bite wounds has been observed in the spring (Gallagher & Nelson 1979), coinciding with a peak in badger mating activity (Cresswell *et al.* 1992; Roper 2010), and territorial behaviour (Kruuk 1978; Roper & Lups 1993). The risk of transmission via this route may therefore be highest at this time.

In addition to direct routes of disease transmission, there is also the potential for infection to spread indirectly between individuals. For example, the badger sett is an environment conducive to the survival of *M. bovis* due to its constant temperature, darkness, and high levels of humidity (Roper 1992). As *M. bovis* can be excreted in urine, sputum and faeces (Clifton-Hadley, Wilesmith & Stuart 1993), any excretions underground may result in a long-term source of infection. Although frequent defecation within the sett occurs rarely, each sett does usually contain at least one latrine (Roper 1992). Therefore, with the poor respiratory conditions experienced underground (Roper 1992), the risk of infection via aerosols from these sources may be high. However, the sett as a source of infection has not been widely addressed, and unless denning preferences and TB status within the sett are connected, they fail to explain how *M. bovis* can remain isolated within pockets of a social group (Delahay *et al.* 2000b).

Latrines may also represent an indirect transmission route of bTB between badgers. Badgers communicate and demarcate their territory boundaries using scent marks at latrine sites (Roper *et al.* 1993). These shallow pits contain badger excretory products and scent marks from faeces, urine, and secretions from the anal, subcaudal and interdigital glands (Delahay *et al.* 2000a), and often contain high concentrations of *M. bovis* (Hutchings & Harris 1999). Therefore, visiting latrine sites may pose an indirect transmission risk to individuals. However, heterogeneities in latrine visitation rates means that only a small proportion of the badger population is likely to be at risk (Drewe *et al.* 2013).

Demographic differences in disease acquisition, progression and survival rates have been observed: male badgers are more likely to acquire bTB infection, will progress through disease states more quickly, and are more likely to die during intermediate stages of infection (Graham *et al.* 2013). It is possible that these sex differences in susceptibility may be due to badger behaviour. For example, male badgers are more likely to move groups, behaviour that is associated with increased disease incidence (Vicente *et al.* 2007). Males also have higher levels of bite wounding (Delahay *et al.* 2006b), which is associated with progressed infection (Jenkins *et al.* 2012). It may be possible that increased bite wounding could be a product of infection, with a decrease in body condition reducing an individual's social status and therefore increasing their exposure to aggression (Jenkins *et al.* 2012). However, social network position is not related to poor body condition or bite wounding (Reed 2011), and instead this sex difference in susceptibility has been attributed to sex differences in immunocompetence, with males investing more in territorial and aggressive behaviour rather than immunological defences (McDonald *et al.* 2014).

Interspecies transmission of bovine tuberculosis

The extent to which badgers contribute to cattle infection is currently unclear, although it has been suggested that badgers are directly responsible for approximately 6% of new herd incident cases, leading to an overall contribution of 50% through subsequent cattle-cattle transmission (Donnelly & Nouvellet 2013). Direct interspecies disease transmission is thought to be low, given how rarely direct badger-cattle contact occurs (Drewe *et al.* 2013; Woodroffe *et al.*

2016). However, indirect transmission is likely to be higher, with cattle known to have high levels of contact with badger latrines (Hutchings & Harris 1997; Drewe *et al.* 2013). In addition, contact with contaminated feed and salt licks pose a further indirect transmission risk to cattle (Benham 1985; Garnett 2002). Therefore, indirect contact via environmental contamination is thought to be the most typical route of disease transmission between badgers and cattle (Figure 1.3).

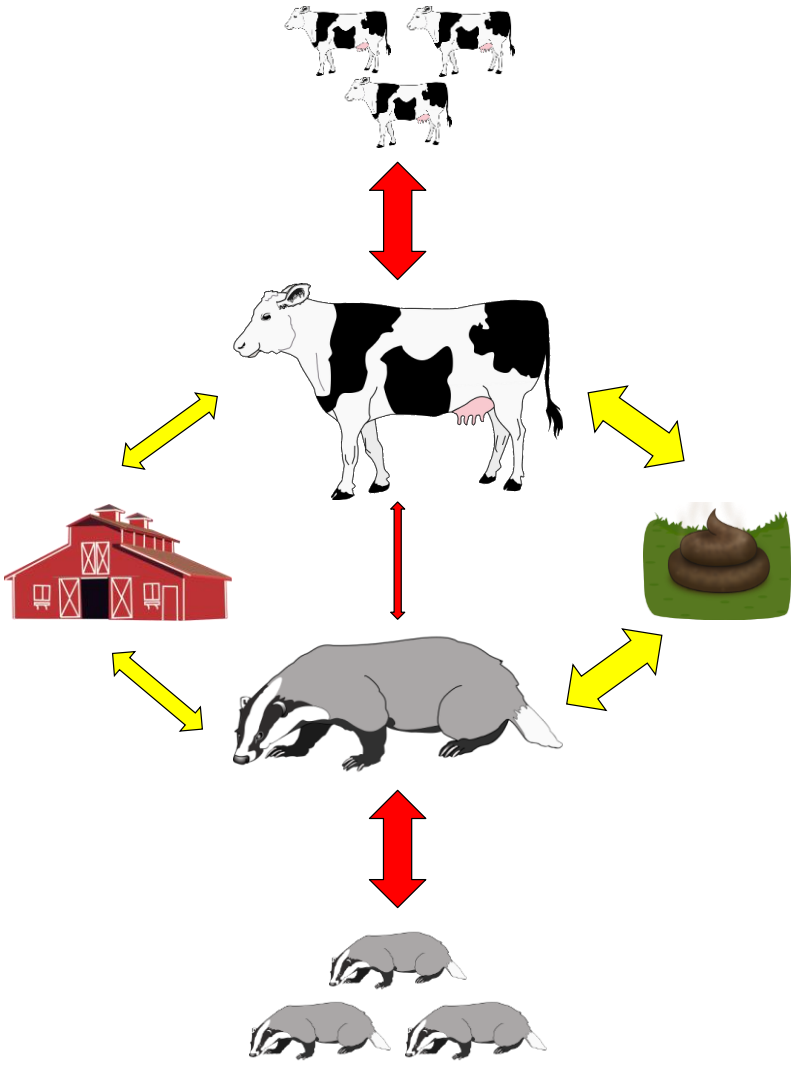


Figure 1.3 A diagram illustrating the typical routes of bovine tuberculosis transmission between badgers and cattle. Red arrows signify transmission through direct contact events, and yellow arrows signify transmission through indirect contact events. Thickness of arrows illustrates the likely frequency of transmission through that route.

In order to control cattle bovine tuberculosis in the UK, badgers have been periodically culled since 1975 using numerous strategies. Between 1975-1982 badgers on land with TB test-positive cattle were tested. If a TB test-positive badger was found the whole sett (the underground burrow where badgers rest during the day) was gassed. This was also occasionally repeated on neighbouring land. This approach to culling was followed by the clean ring strategy between 1982-1986, in which badger groups were removed from land with a herd breakdown (i.e. a bTB outbreak), continuing outwards until a 'clean ring' of TB test-negative badger groups had been removed. Between 1986-1997 the interim strategy removed badgers that could be trapped on the same land that the breakdown occurred, or the whole farm if this could not be identified (Krebs *et al.* 1997).

In 1997 an experimental cull was initiated to determine the full effects of badger culling on the incidence rates of bTB in cattle. The randomised badger culling trial (RBCT) trialled 3 badger management strategies: reactive culling where badgers in surrounding areas were culled in response to a herd breakdown, proactive culling where badgers were repeatedly culled regardless of herd breakdowns, and control areas where badgers were not disturbed (Krebs *et al.* 1997). Of these three, reactive culling was most similar to previous management strategies. Counter to expectation, cattle TB incidence within the culled area was found to increase in response to reactive culling. Therefore this element of the trial was terminated early (Donnelly *et al.* 2003). Proactive cull areas showed a decrease in bTB incidence in cattle, however the areas surrounding the proactive cull zone showed an increase in herd incidents, neutralising any positive effect proactive culling had within the cull zone (Donnelly *et al.* 2006). Therefore, whether the positive effects that badger culling could have for cattle breakdown rates justifies the substantial effort and financial costs required to achieve them has been questioned (Donnelly *et al.* 2007; Jenkins *et al.* 2007). This counterintuitive effect of culling on TB incidence could be explained by the effect culling had on the social structure of badger populations (Tuyttens, Macdonald & Long 2000; Woodroffe *et al.* 2006, 2008; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011; Bielby *et al.* 2014).

Badger social structure and bovine tuberculosis

At medium to high densities, badgers live in mixed-sex groups of up to 35 individuals, with an average of 5.2 (Da Silva *et al.* 1994; Tuyttens *et al.* 2000; Johnson, Jetz & Macdonald 2002). Together, individuals within these groups defend communal territories which can remain stable over many years (Kruuk 1978). Analysis of badger contact data has shown that individuals mostly interact with members of their own group (~90% of contacts) (Goodman 2007; Reed 2011), but contact rates do vary between individuals (Böhm, Hutchings & White 2009). Radio tracking studies have shown that this variation can lead to asymmetries within the badger contact structure, which may be related to season and space use (Böhm *et al.* 2008). Extra-group contact also occurs during foraging, aggressive encounters and extra-group mating (Roper 2010), but almost exclusively (~97% of extra-group contacts) occurs between neighbouring groups (Goodman 2007; Reed 2011). This stable badger social structure with limited among-group contact is thought to restrict the transmission of disease between groups (Delahay *et al.* 2000b).

Artificially reducing badger densities caused this stable badger social structure to breakdown. In response to culling, badgers increased their home range size, had less defined territories, had greater home range overlap with neighbouring groups, and had increased movement among groups (Tuyttens *et al.* 2000; Woodroffe *et al.* 2006, 2008; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011; Bielby *et al.* 2014). This was found to directly increase TB prevalence within the badger population, likely from an increase in contact rates and/or stress induced immunosuppression (Riordan *et al.* 2011) reducing the overall spatial clustering of bTB infection (Jenkins *et al.* 2007). This effect, termed ‘social perturbation’, demonstrates the importance of badger community structure and behaviour for bTB disease dynamics (Carter *et al.* 2007; McDonald *et al.* 2008).

Subsequent studies have found further links between bTB infection and badger behaviour. Analysis of capture-mark-recapture data has revealed that the movement of badgers between groups is associated with an increase in TB incidence (Rogers *et al.* 1998; Vicente *et al.* 2007). It may be possible that this behaviour is related to breeding activity, given that males are more likely to

move groups, particularly to those with a high proportion of females (Rogers *et al.* 1998). In addition, studies on badger contact data have revealed that an increase in among-group contact rates coincides with a known peak in badger sex hormone levels, and males have higher among-group flow-betweenness scores during mating seasons (Reed 2011). Extra-group contact events between males and females are also known to peak at these times (Goodman 2007). Therefore, it is possible that badger mating behaviour is driving these among-group interactions that are likely to be important for disease transmission.

Social network analysis has also revealed that network position is related to TB test-outcome; in the autumn and winter, TB test-positive badgers were found to have less contact with individuals from their own group, but were more important in connecting social groups in the summer and winter (Weber *et al.* 2013a). This suggests that infected individuals will be less influential for disease spread within their own groups, but more important in transmitting disease among groups. This important position in bridging sub-groups will reduce the protection group living can offer against disease spread (Wu & Liu 2008; Jones & Salathe 2010).

TB test-positive badgers are also known to differ in their patterns of sett use. Setts are underground burrows where badgers rest during the day. They are broadly split into two categories: main setts and outlier setts. Typically main setts are large, have many entrance holes and chambers, and are normally permanently inhabited (Kruuk 1978). Outlier setts tend to be smaller, are disconnected from the main sett, have only one or two entrance holes and are only intermittently used (Kruuk 1978). TB test-positive badgers were found to consistently use outlier setts more frequently than TB test-negative badgers (Weber *et al.* 2013b). In addition, TB test-positive badgers have larger ranges that overlap more with neighbouring groups (Cheeseman & Mallinson 1981; Garnett, Delahay & Roper 2005). Therefore it has been theorised that these behaviours are linked, with outlier sett use facilitating extra-territorial forays, and therefore extra-group contact (Weber *et al.* 2013a). However the relationships between sett use and ranging behaviour, and ranging behaviour and social network position, have yet to be explored.

Traditional disease models that assume badger contact rates to be homogenous have not captured the importance of badger social structure for the spread of bTB. Use of these models has led to the erroneous prediction that a reduction in badger densities would suppress disease prevalence in cattle (Anderson & Trewhella 1984). Subsequent models have attempted to address these assumptions of homogenous mixing, by including variation in within- and among-group disease transmission rates (White & Harris 1995a). Deemed to be a more realistic representation of disease transmission in badger populations, this type of model has also been used to test the efficacy of different control strategies (White & Harris 1995b). However, empirical data on the within- and among-group contact rates for this model were unavailable, and so typical contact rates were estimated based on historic observation studies (White & Harris 1995a). Given the evident heterogeneity in badger behaviour and population contact structure that will likely influence disease transmission, it is clear that badger contact structure must be fully understood to effectively study the spread of bTB.

1.4 This thesis

This thesis aims to explore different elements of community structure within a badger population, and the implications of this structure for bTB transmission. In order to achieve this, data from the Woodchester Park badger population is used. The Animal and Plant Health Agency (APHA) have studied this population since 1976, facilitating the collection of a variety of data. The data used in this thesis are outlined in the following section and summarised in Table 1.1.

Data overview

At Woodchester Park, bait-marking data is collected annually to allow the configuration of badger social groups to be determined. Baits are placed at badger setts containing unique indigestible pellets, which can then be identified in badger defecations (Delahay *et al.* 2000a). During surveys, the location that each type of pellet was retrieved from is recorded. Because badgers mark their territorial boundaries with latrines (Roper *et al.* 1993), this information can be used to determine the territory boundaries of each badger social group. Bait marking data from 2005-2011, and 2014 are used in this thesis.

The Woodchester Park badger population is also the subject of a long-term capture-mark-recapture (CMR) study. At first capture, each badger is marked with a unique tattoo, allowing them to be identified when subsequently caught. At every capture event, information including the individuals trapping location, age, sex, and weight are recorded. Samples for bTB testing are also taken. Trapping events at Woodchester Park are carried out four times a year, allowing a capture history for each individual caught in the population to be created. Data for selected badgers that were caught between 2005-2009, and 2014-2015 are used in this thesis.

When badgers are first captured, hair samples are taken and submitted for DNA extraction and genotyping. This information is used to calculate the relatedness of each pair of individuals in the population. For this thesis, relatedness estimates for selected individuals caught between 2009-2010 are used.

Trapping also provides an opportunity to collar individuals. For this thesis, badgers were collared with proximity loggers, which allow high-resolution contact data to be recorded. These data are used to build contact matrices based on either the duration or frequency of interactions that occur between individuals, and is analysed using social network analysis. The proximity data used in this thesis were collected between 2009-2010, and 2014-2015.

Proximity collars also contain VHF transmitters, enabling collared badgers to be radio tracked concurrently to their proximity information being recorded. Badgers were diurnally radio tracked to enable their daytime resting locations to be determined, and if they were resting in a main or an outlier sett. The sett use data used in this thesis were collected over 28 consecutive days in four seasons between 2014-2015.

Badgers were also nocturnally radio tracked to allow data on badger ranging behaviour to be collected. Badgers were radio tracked concurrently to being diurnally radio tracked across three seasons (autumn, winter, spring) between 2014-2015. These locational data are used to calculate badger home range size per season, and also the proportion of time that badgers were located in other group territories.

Table 1.1 Summary of datasets used in this thesis

Dataset	Brief description	Date collected	Chapter
Bait Marking	Spatial configuration of badger social groups per year. Contains the location of each bait return.	2005-2011 & 2014	2, 3, 4, 5
Capture-Mark-Recapture	Dataset containing details of badger capture location, sex, age and disease status	2005-2009 2014-2015	2, 3, 4, 5
Relatedness	Matrix of relatedness between pairs of individuals in the population.	2009-2010	3
Proximity	Matrix of the duration or frequency of interactions that occurred between pairs of collared individuals	2009-2010; 2014-2015	2, 3, 4, 5
Sett use	Diurnal resting locations of collared individuals.	2014-2015	4
Home Range	Nocturnal locations of collared individuals.	2014-2015	4 & 5

Chapter overview

The datasets outlined in the previous section (bait marking, CMR, relatedness, sett use, and home range) are used in this thesis to explore the following themes:

In chapter two, I identify the community structure of the badger population using social network analysis. Given the spatial clustering of bTB infection within the badger population (Delahay *et al.* 2000b), I expect to detect a very strong community structure. I also explore how individuals contribute to this structure, allowing individuals that bridge these communities to be identified, and their implications for disease transmission discussed. TB test-positive badgers are expected to significantly contribute to this structure, given that they are known to be important in connecting badger social groups (Weber *et al.* 2013a). Previous studies have used an established method, based on the retrieval of marked baits from latrines, to identify badger social structure (Delahay *et al.* 2000a). However, high levels of extra-group contact between some groups (Reed 2011) may indicate that the social communities badgers interact in differ to those identified using spatial cues. Therefore, different methods of community detection are also compared. This chapter uses the CMR, bait marking and the 2009-2010 proximity datasets. The communities identified in this chapter are used in all subsequent analyses in this thesis.

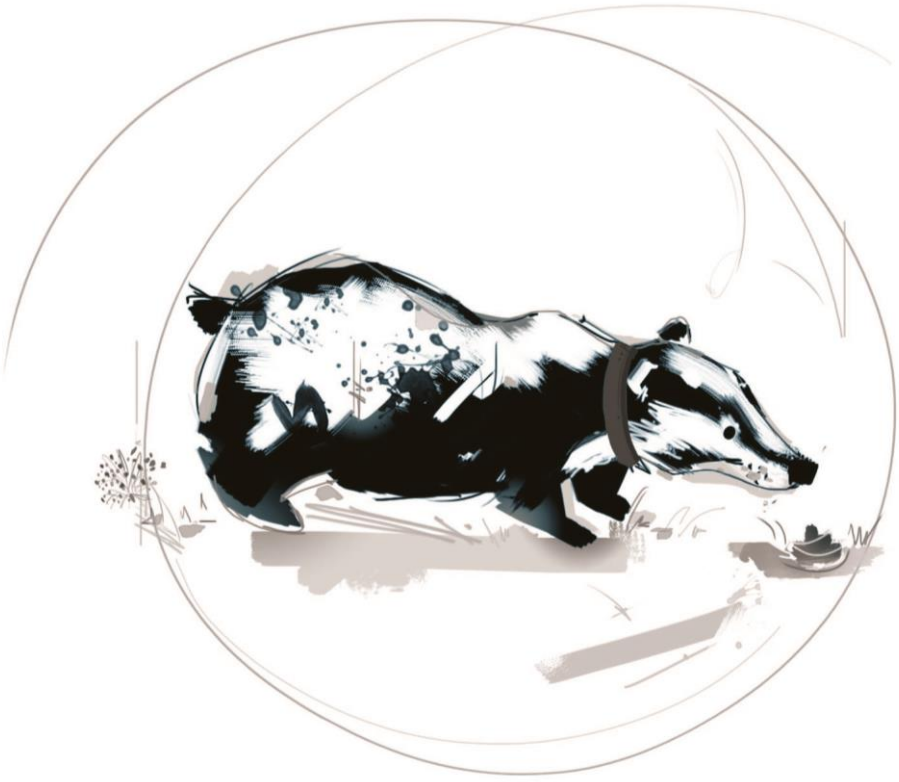
In chapter three, I explore the role of relatedness in explaining badger contact rates, and determine if individuals mix assortatively with relatives. Given that this type of mixing has been observed in other systems (e.g. Widdig *et al.* 2001; Griffiths & Armstrong 2002; Archie, Moss & Alberts 2006; Gero, Engelhaupt & Whitehead 2008; Wiszniewski, Lusseau & Möller 2010), badgers are expected to spend more time with those that they are related to. The extent to which relatedness can explain extra-group contact events will also be explored, with relatives expected to spend less time together, given that badgers are thought to seek extra-group mating for reasons related to inbreeding avoidance (Carpenter *et al.* 2005; Annavi *et al.* 2014). The implications of these extra-group contacts for disease spread to the wider population are also discussed. This chapter uses the CMR, bait marking, relatedness and 2009-2010 proximity datasets.

In chapter four, I use home range and sett use data to determine if outlier sett use facilitates extra-territorial forays, and in chapter five I determine if badgers that partake in extra-territorial forays hold different positions in the network. How these behaviours and the relationships between them vary across the year is also determined, to improve the understanding of the seasonality of badger behaviour and the implications this might have for disease transmission. These two chapters will give insight into behaviours that are likely to connect communities in the population, and therefore be important for disease spread. TB test-positive badgers have previously been observed to use outlier setts more frequently, have larger home ranges, and be important in connecting social groups (Cheeseman & Mallinson 1981; Garnett *et al.* 2005; Weber *et al.* 2013a; Weber *et al.* 2013b). Therefore, outlier sett use is expected to be associated with larger home ranges and greater home range overlap, which subsequently is expected to be associated with more central network positions. Whether TB test-positive badgers are associated with these behaviours is also determined. How this information might be used to advise disease control strategies is also discussed. These chapters use the CMR, bait marking, sett use and home range datasets, and the 2014-2015 proximity dataset.

Finally, in chapter six, I discuss the implications of these results for the epidemiology and control of bovine tuberculosis in badgers. The applicability of

these findings to disease transmission in general will also be explored, and avenues for future research discussed.

Chapter 2
Community Detection in a Population of Badgers



2.1 Abstract

1. The social clustering of individuals into separate communities can strongly affect the spread of infection. Therefore, effective identification of the community structure of a population can increase the understanding of disease dynamics.
2. This study aimed to quantitatively determine the community structure of a European badger (*Meles meles*) population, and calculate the extent to which individuals contribute to this structure. Badger social structure is thought to limit the spread of bovine tuberculosis (bTB), of which badgers are a wildlife reservoir, with TB test-positive individuals known to be important in connecting groups. Therefore, I expected to find a very strong community structure, with TB test-positive badgers anticipated to significantly contribute to this structure. This study also aimed to compare different methods of community detection.
3. By using empirically derived contact data, I used social network analysis to directly identify the community structure of the badger population. I compared this structure to the communities identified using an established, indirect method that is based on the retrieval of marked baits from latrines.
4. Individuals were found to interact in fewer, more distinct social groups than had previously been assumed using the bait marking method. Individual contribution to community structure was found to vary with sex and age, with sub-adults and males increasing the isolation of communities. However, TB test-positive individuals did not influence community structure, despite being important for flow among groups.
5. The discovery of these highly distinct communities can help explain why badger social structure can inhibit the spread of disease through the population. However, this also has important implications for the study and understanding of disease dynamics, where the reliance on bait marking may mask true patterns of social interactions. I recommend that future studies concerning disease transmission consider using network methods for community detection, where possible.

2.2 Introduction

Disease transmission can be strongly affected by variation in contact rates between individuals (Wey *et al.* 2008). This variation can result in some individuals being more connected than others within a single population (Palla *et al.* 2005). The effect of this clustering has been extensively explored in network theory. For example, high local clustering in lattice networks slows disease spread, causing transmission to occur in waves across the population (Keeling & Eames 2005). Small world networks are also highly clustered, but the addition of rare, long-range contacts creates a network that is both globally connected and locally clustered (Keeling & Eames 2005). This structure allows disease to spread quickly through the entire population, making individuals that connect these clusters important for disease dynamics. High levels of clustering can lead to the formation of distinct social communities, where members interact more with each other than with the rest of the population (White & Harris 1994; Palla *et al.* 2005; Böhm *et al.* 2008). The size and connectivity of these communities can influence disease spread, with small, isolated groups having a lower infection risk (Altizer *et al.* 2003; Cross *et al.* 2004). For example, Tasmanian devils (*Sarcophilus harrisii*) have limited community structure, with all individuals in a population effectively belonging to a single group (Hamede *et al.* 2009). As in lattice networks, this means every individual could share similar risks of infection if new diseases enter the population (Hamede *et al.* 2009). However, even if individuals spend part of their time in distinct social groups, temporal variation in these associations and/or high levels of movement between these groups can markedly affect infection dynamics. For example, meerkat (*Suricata suricatta*) social groups are connected by roving individuals that are more likely to acquire infection (Drewe 2010), and movement between European badger (*Meles meles*) social groups has been associated with an increase in disease incidence (Rogers *et al.* 1998; Vicente *et al.* 2007).

An individual's contribution to community structure can vary. For example, individuals that commonly interact with their own social group and rarely with others will increase the number of within-group edges, leading to communities becoming more isolated (Clauset, Newman & Moore 2004). However, those that commonly seek extra-group interactions will increase the number of

among-group edges, and make these communities less distinct (Clauset *et al.* 2004). This can be seen in male urban red foxes (*Vulpes vulpes*) that frequently enter neighbouring territories to seek extra-group mating (White & Harris 1994). If these individuals that weaken community structure can be identified, this could inform epidemiological analyses and, potentially, allow the development of more targeted approaches to disease control (Bascompte 2007).

In mammals, spatial information such as the location of scratch and scent marks can be utilised in order to determine territorial structures. Although some species scent mark individuals to identify fellow group members (Johnson 1973), other species scent mark throughout their territorial range to communicate spatial ownership of an area (Johnson 1973; Peters & Mech 1975; Jordan, Cherry & Manser 2007). These scent marks are often left at latrine sites, which can be useful indicators of territorial boundaries due to their conspicuous nature (Peters & Mech 1975; Erlinge, Sandell & Brinck 1982; Roper, Shepherdson & Davies 1986; Sillero-Zubiri & Macdonald 1998). Many individuals visit latrines, particularly at boundaries where their scent is used to demarcate social group territories (Johnson 1973). Therefore, if these latrines can be attributed to specific social groups, then the social group membership of individuals living within these areas can be inferred. This method, known as bait marking, is commonly used when studying European badgers (Delahay *et al.* 2000a). Alternatively, social network analysis can define community structure directly through the use of empirically derived contacts, rather than spatial information (Wey *et al.* 2008). These contacts can be recorded through directly observing individuals, and has been used successfully in studies of dolphins (Lusseau *et al.* 2006), brent geese (Silk *et al.* 2015) and field cricket populations (Fisher, Rodríguez-Muñoz & Tregenza 2016). Directly observing individuals can be a laborious process and is inappropriate for studying elusive species; however, technological advances have helped to overcome these issues. For example, proximity loggers can record interactions between individuals in a population, and social network analysis can be performed to directly identify social groups. These loggers have already been used with success to investigate contact rates between badgers, and between badgers and cattle, to give insight into the epidemiology of bovine tuberculosis (Böhm *et al.* 2009; Drewe *et al.* 2012; Weber *et al.* 2013a).

Bovine tuberculosis (bTB) is a chronic, debilitating disease of cattle caused by the bacterium *Mycobacterium bovis*. Globally, this disease causes great economic loss through reduced productivity, loss of trade, and compensation pay-outs (Michel *et al.* 2009). In developed countries, bTB is largely managed in cattle through extensive test-and-slaughter programmes and restrictions upon herd movements (Krebs *et al.* 1997; Michel *et al.* 2009). However, the success of this method can be compromised when wildlife disease reservoirs are present, with disease control in the UK complicated by the discovery that badgers can carry bTB in 1971 (Muirhead *et al.* 1974). At medium to high densities these social mammals typically live in mixed-sex groups that defend stable territories (Tuyttens *et al.* 2000). Badger social structure has previously been shown to influence bTB dynamics both in badgers and in cattle. For example, movement among groups has been associated with an increase in disease incidence in badgers (Rogers *et al.* 1998; Vicente *et al.* 2007), and increased dispersal rates, range sizes, and social group overlap following a cull (Woodroffe *et al.* 2006; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011) have been associated with increases in bTB incidence in cattle (Donnelly *et al.* 2003, 2006; Woodroffe *et al.* 2006). Individuals have also been shown to vary in their network position, with TB test-positive badgers found to be important in linking social groups (Weber *et al.* 2013a).

This study aims to use a social network approach to quantitatively determine the community structure of a wild badger population. As previous studies suggest that stable social groups may inhibit the spread of bTB in badger populations (Tuyttens *et al.* 2000; Delahay *et al.* 2000b), highly distinct communities are expected to be found. How contribution to community structure is related to specific traits, such as age, sex and disease status, is also determined. It is predicted that diseased badgers will weaken the structure of the network, resulting in less distinct communities that are linked through these infected individuals. The direct social network method of community detection is compared to an indirect method - the traditional bait marking approach that has commonly been used to identify badger social structure (Delahay *et al.* 2000a). This will enable the relevance of the bait marking method to be determined in the context of disease transmission. Finally, to determine how the choice of community detection method influences results, an existing analysis that

determines the network position of TB test-positive badgers both within and among groups is repeated using groups identified using both the bait marking and social network approaches.

2.3 Methods

Study Site

Woodchester Park (N51°42' 34", W2°16' 26") is situated on the Cotswold limestone escarpment in Gloucestershire, South West England. The core study area of 7km^2 comprises of mixed woodland, pasture and arable farmland, and has a resident high-density badger population that is the subject of a long-term capture-mark-recapture study (Delahay *et al.* 2000b, Figure 2.1).

Badger sampling

Trapping events at Woodchester Park are carried out approximately 4 times a year using methods described in Delahay *et al.* 2006a. Briefly, badgers were caught using steel mesh cage traps that are baited with peanuts. They were then anaesthetised using an intramuscular administration of two parts butorphanol tartrate (Torbugesic®, Wyeth, Ontario, Canada), two parts ketamine hydrochloride (Ketaset®, Wyeth, Ontario, Canada) and one part medetomidine (Domitor®, Orion Corporation, Espoo, Finland) (De Leeuw *et al.* 2004). The capture location, sex, age and infection status were then recorded for each individual. Age is categorised as either sub-adult (>1 and <2.5 years), or adult (>=2.5 years) (Weber *et al.* 2013b). Individuals were considered TB positive if they reacted positively to either of two diagnostic tests. These were the badger-specific lateral flow antibody immunoassay (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, NY, USA), and an enzyme immunoassay for interferon-gamma (IFNγ) production in response to stimulation with purified protein derivatives of *M. bovis* and *M. avium*. When serological and cytokine assay results were combined, the sensitivity and specificity of the combined test were at least 85% and 93% respectively (Dalley *et al.* 2008; Chambers *et al.* 2009). Fifty-one badgers that were captured as part of this long-term study were collared with proximity loggers (Sirtrack, New Zealand) over 11 days between May and October 2009. The age, sex, disease status and capture locations of these individuals are summarised in Table 2.1 and Figure 2.1.

Contact events

Proximity collars deployed on 51 badgers recorded contact data between May 2009 and May 2010. These collars contained an Ultra High Frequency (UHF) transceiver that broadcasts a unique ID code whilst simultaneously 'listening' for those of others (Drewe *et al.* 2012). When loggers came within a defined distance, a contact was initiated until a signal could no longer be detected (Drewe *et al.* 2012). Details of the interaction were then recorded on-board the collar (Drewe *et al.* 2012). These loggers were individually set to record interactions when within 0.64+/- 0.04m of another collared individual (UHF range 34-48). This enabled interactions that occurred at close distances (e.g. fighting, grooming and mating) to be recorded, although different types of interaction cannot be differentiated in the data. Of the 51 badgers collared, 11 had collars that were not retrieved. This was due to either the collar being dropped underground, or the badger not being recaptured at the end of the study. However, contact data were downloaded whenever badgers were recaptured throughout the year. All contacts that occurred during trapping operations and the 12 hours following were deleted to allow normal behaviour to resume. Proximity loggers have the tendency to record extended interactions as a series of shorter contacts (Drewe *et al.* 2012). Therefore, to improve the accuracy of the interaction data the protocols suggested by Drewe *et al.* 2012 were followed; all interactions that were recorded within 1.5 minutes between the same pair of loggers were amalgamated, and any additional 1-second interactions were removed.

Territory mapping using bait marking

Badger latrines are shallow pits in which excretory products are deposited along with scent marks from faeces, urine, and secretions from the anal, subcaudal and interdigital glands (Delahay *et al.* 2000a). Bait marking in the population studied is carried out each spring when latrine use is at its peak (Kruuk 1978), making territory boundaries easier to identify. This method is described in detail in Delahay *et al.* 2000a. Briefly, each territory typically has an associated main sett which is usually permanently occupied, and used by all members of a social group (Roper 1992). Baits containing small coloured indigestible plastic pellets were placed at putative main setts, with a differently coloured or shaped pellet used at each. The setts targeted for this study are shown in Figure 2.2. Pellets

were then recovered from badger faeces during surveys for latrines. This method allowed the use of each latrine to be attributed to a specific sett. Bait return data were used alongside field observations, such as well-used badger paths between adjacent territories, to infer territory boundaries. The territories identified are thought to give information on the configuration of badger social groups within the population (Delahay *et al.* 2000a). The location where each individual is trapped in relation to the mapped territorial boundaries is used to determine the advertised group to which each badger belongs.

Advertised territories were mapped using the bait marking method between 2008 and 2011. However, any bait returns that are recovered from outside these territory boundaries are regarded as outliers from extra-territorial forays (Delahay *et al.* 2000a), suggesting that territory boundaries are not necessarily definitive. Therefore, in order to assess the level of certainty associated with each territory boundary per year, a Simpson's Diversity Index was calculated. This index captures the number of bait returns that fell outside the group territory boundary, and also the number of different territories they were recovered from. This would indicate if some territory boundaries are more distinct than others, and if some territories are more linked to adjacent territories. The Simpson's Diversity Index is calculated using the following equation:

$$D = 1 - \sum \left(\frac{n}{N}\right)^2$$

Where *n* denotes the number of bait returns found in each territory and *N* is the total number of bait returns. Values ranged from 0 to 1, with 0 indicating no diversity with all bait returns recovered from a single social group, and 1 indicating an infinite diversity of bait recovery locations.

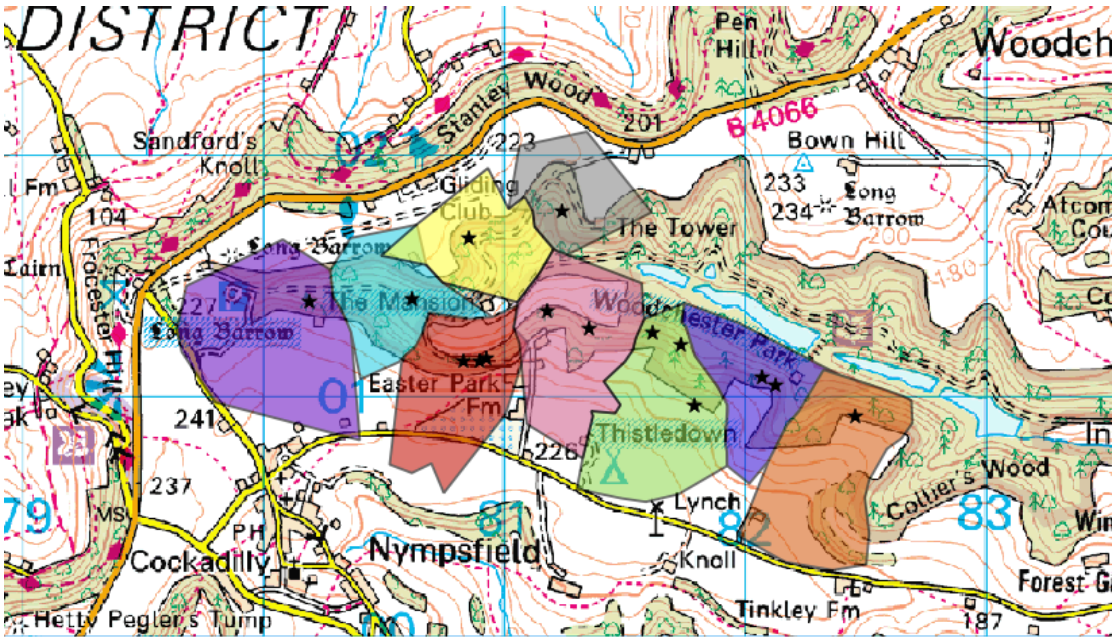


Figure 2.1 Capture locations (black stars) of the 51 badgers collared in 2009 and their associated advertised territory boundaries determined using bait marking (polygons). Polygon colour represents the following badger groups: Purple = West, turquoise = Larch, red = Beech, yellow = Cedar, grey = Boxwood, pink = Septic Tank, green = Top/Yew, blue = Wych Elm, orange = Kennel.

Table 2.1 The demographic classes of the 51 collared badgers. Advertised group membership was determined using badger capture location and bait marking data. Functional group membership was determined using modularity analysis.

Functional social group	Advertised spatial group	Sex		Age		Infection status	
		Male	Female	Adult	Sub-adult	Positive	Negative
Group 1	West	3	2	3	2	3	2
Group 2	Larch	3	2	3	2	2	3
Group 3	Beech	4	5	4	5	3	6
Group 4	Cedar	2	2	3	1	3	1
	Breakheart	0	1	1	0	1	0
	Septic Tank	1	1	0	2	2	0
Group 5	Top/Yew	6	6	8	4	3	9
Group 6	Wych Elm	5	3	4	4	3	5
	Kennel	0	5	1	4	1	4

Figure 2.2 Map of Woodchester Park, the site of a long-term capture-mark-recapture study of the resident high-density badger population. Selected active main setts targeted with bait to reveal advertised territory boundaries are indicated by black stars.

Community detection using the social network approach

Social network analysis is a quantitative tool to analyse social structure (Hawe *et al.* 2004). Networks consist of nodes (individuals) that are connected by edges (interactions), which can be binary to represent the presence or absence of interactions, or weighted to illustrate the frequency or duration of interactions. This simple representation allows many parameters to be estimated, giving a greater insight into the population than could be attained from analysing individuals in isolation (Hawe *et al.* 2004). In this study, social networks were built from the 2009/2010 proximity logger contact data from all 51 badgers collared, using an R script which amalgamates contacts into a matrix ready to be analysed (Reed 2011).

To identify community structure within the badger network, I calculated the network modularity (Q) using the R package 'igraph' (Csardi & Nepusz 2006). This metric is defined as the fraction of within-group edges in the observed network minus the expected fraction of within-group edges in a randomised null model (Newman 2006). This null model is based on the observed network

graph, but rearranges the edges randomly with no regard to community structure (Newman 2006). Q is calculated using the following equation:

$$Q = \frac{1}{2m}\sum_{ij}\left[A_{ij} - \frac{k_i k_j}{2m}\right]\delta_{gigj}$$

(Newman 2011)

Where the expected number of edges falling between two vertices (*i* and *j*) is equal to $k_i k_j/2m$, where *k* is the node degree and *m* is the total number of edges in the observed network. The actual number of edges observed between two nodes is equal to A_{ij}. An integer label is given to each node denoting the group it belongs to in the proposed network division (g_i), and δ_{ij} is the Kronecker delta which tests whether the nodes belong to the same group (Newman 2011).

By comparing the observed to the randomised networks it can be determined whether the number of within-group edges is greater than would be expected by chance (Newman 2006), and gives a Q value that can range from -1/2 to 1. If Q<0 then no community structure is identified, but if Q=1 then highly structured communities have been detected (Newman & Girvan 2002). Community structure is generally regarded to be present if Q>0.3 (Clauset *et al.* 2004).

Q was fully optimised to identify divisions in the network, and the communities present (Newman 2006). This was done by first placing each individual in a separate community and calculating the modularity. Neighbouring communities were then joined to produce the largest increase in modularity possible. This process was repeated until the pattern of network division that gave the highest modularity score was found (Clauset *et al.* 2004; Blondel *et al.* 2008; Verdolin, Traud & Dunn 2014). This approach is referred to as the 'unrestricted network approach' and assigned each individual to a functional social group. Q was then calculated for the community structure identified using the 2009 bait marking data, and assigned each individual to an advertised spatial group. Finally, Q was partially optimised using a 'restricted network approach', to determine the best possible split of the network given the same number of groups as when determined by bait marking.

All analyses took into account the edge weight, which was taken as the frequency of interactions, and were carried out using both the multi-level community and fast-greedy functions in the R package 'igraph' (Csardi & Nepusz 2006). Both functions gave the same result. The three resulting network divisions (fully optimised unrestricted, bait marking, partially optimised restricted) were drawn using the software Netdraw (Borgatti, Everett & Freeman 2002).

Comparing methods of community detection

Confidence intervals for the three modularity estimates (calculated for each method of community detection: the fully optimised unrestricted network approach, the bait marking approach, and the partially optimised restricted network approach) were calculated using the bootstrapping method described in Lusseau, Whitehead & Gero 2008. To do this, edges from the network were resampled with replacement to create 1000 varying networks of equal weight.

Bootstrapping was repeated for differently weighted networks to determine how weight influences the identification of community structure. These were the network weighted by interaction frequency, log(frequency+1), and binary. The weighted network allows common within-group contacts to be distinguished from rare among-group contacts. The log(frequency+1) network will reduce the influence of the most frequent interactions. Finally the binary network treats the most frequent interactions the same as the most rare.

Calculating individual contribution to community structure

An approach similar to the de-lifing method used by Coulson *et al.* 2006 was used to determine the degree to which an individual contributes to the overall community structure of the population. This approach removed an individual from the network and re-calculated Q using the fully optimised unrestricted network approach for the remaining network. This new Q value was then subtracted from the original estimate to give a score reflecting that individual's "contribution" to community structure. To determine which traits influence variation in contribution to community structure, these estimates were modelled against age, sex, bTB infection status and the two-way interactions between these variables in a linear mixed effects model using the R package 'lme4'

(Bates *et al.* 2014). The territory each badger was caught in, identified using the bait marking method, was also included as a random effect to account for any spatial differences in the population.

Competing models were then ranked using AIC values using the R package 'MuMIn' (Bartoń 2013). The parameter estimates and errors were then averaged across the entire candidate set, with each averaged parameter estimate weighted so those with low weights contribute little to the estimate (Symonds & Moussalli 2011).

The implications of method choice

Finally, to determine how the method of community detection can influence results, the analysis from Weber *et al.* 2013a was repeated using two approaches of community detection. The complete network was divided into within- and among-group contacts identified using both the original bait marking and the fully optimised unrestricted social network approaches. Three metrics were then calculated for each network in each season (summer: Jun-Aug 2009, autumn: Sep-Nov 2009, winter: Dec 2009-Feb 2010, and spring: Mar-May 2010). These were degree (duration of contacts directly connected to the individual), closeness (distance of an individual to all others), and flow-betweenness (a measure of positional advantage in the 'flow' across the network, specifically the contribution of a given individual to all possible pathways connecting all pairs in the network). Degree and closeness were calculated in the R package 'tnet' (Opsahl 2009), and flow-betweenness was calculated in the social network software UCINET (Borgatti *et al.* 2002). To determine if these metrics differed for TB test-positive and TB test-negative individuals, UCINET was also used to run node-level t-tests. As network data are non-independent (Croft *et al.* 2011), this analysis uses permutation tests to generate sampling differences between the two means.

2.4 Results

Community detection using bait marking approach

Nine social groups were identified in 2009 using the bait marking method (Figures 2.3 and 2.4A). Variation in group territory boundaries between 2008-2011 was evident, with groups splitting and merging, but the configuration of

group territories generally remaining stable (Figure 2.3, Table 2.2). Frequent changes in boundaries between certain groups across years suggest that some groups are more linked to each other than others in the population (Figure 2.3, Table 2.2).

Community detection using fully optimised unrestricted social network approach

The badger population was structured into six highly distinct groups (mean modularity = 0.732 [95% CI 0.730, 0.733], Figures 2.4B and 2.5). This finding was robust to varying edge weights, however estimates of community structure for the log and binary networks were lower and estimated with less confidence than the fully weighted network (log(frequency+1), mean modularity = 0.674 [0.654, 0.692]; binary mean modularity = 0.484 [0.443, 0.521]; Figure 2.5).

Table 2.2 Simpsons diversity index of each social group's bait returns between 2008-2011. Social groups are numbered 1-10, as per Figure 2.3. Larger values indicate a greater diversity of territories that baits were recovered from. Combined cells indicate social groups that have temporarily merged.

Year	Social Group									
	1	2	3	4	5	6	7	8	9	10
2008	0.19	0.32	0.1	0	0.15	0.28	0.09		0	0.11
2009	0	0	0.24	0.33	0	0.20	0.24		0	0
2010	0	0.45	0.14	0.28	0.31	0.34		0.15	0	0.37
2011	0	0	0.06		0	0.17	0	0.06		0.09

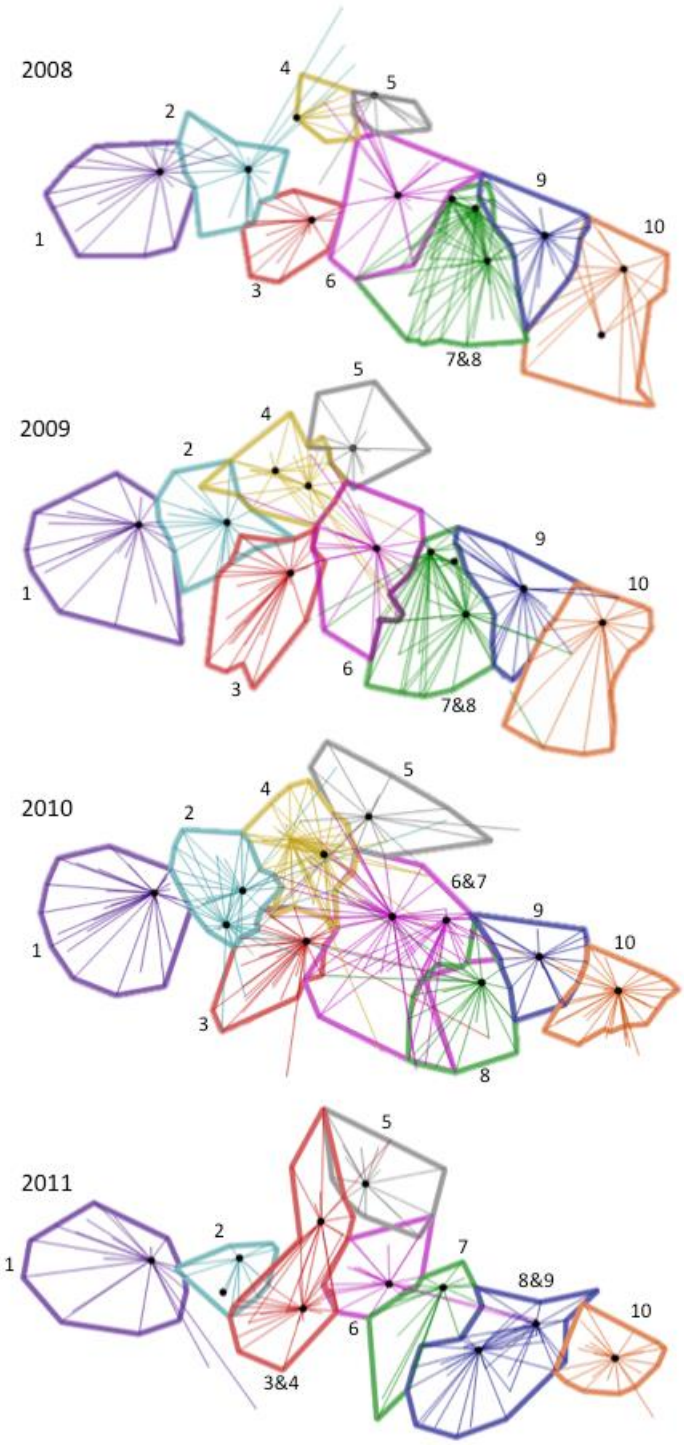


Figure 2.3 Bait marking maps showing the social group boundaries of a population of wild badgers between 2008-2011, using the bait marking method of community detection. Main setts (marked with black circles) are connected to their corresponding latrine, identified using coloured markers in baits fed at each sett. Territory boundaries are determined using minimum convex polygons of the outermost returns, supplemented by field observations of distinct boundary runs (Delahay *et al.* 2000a).

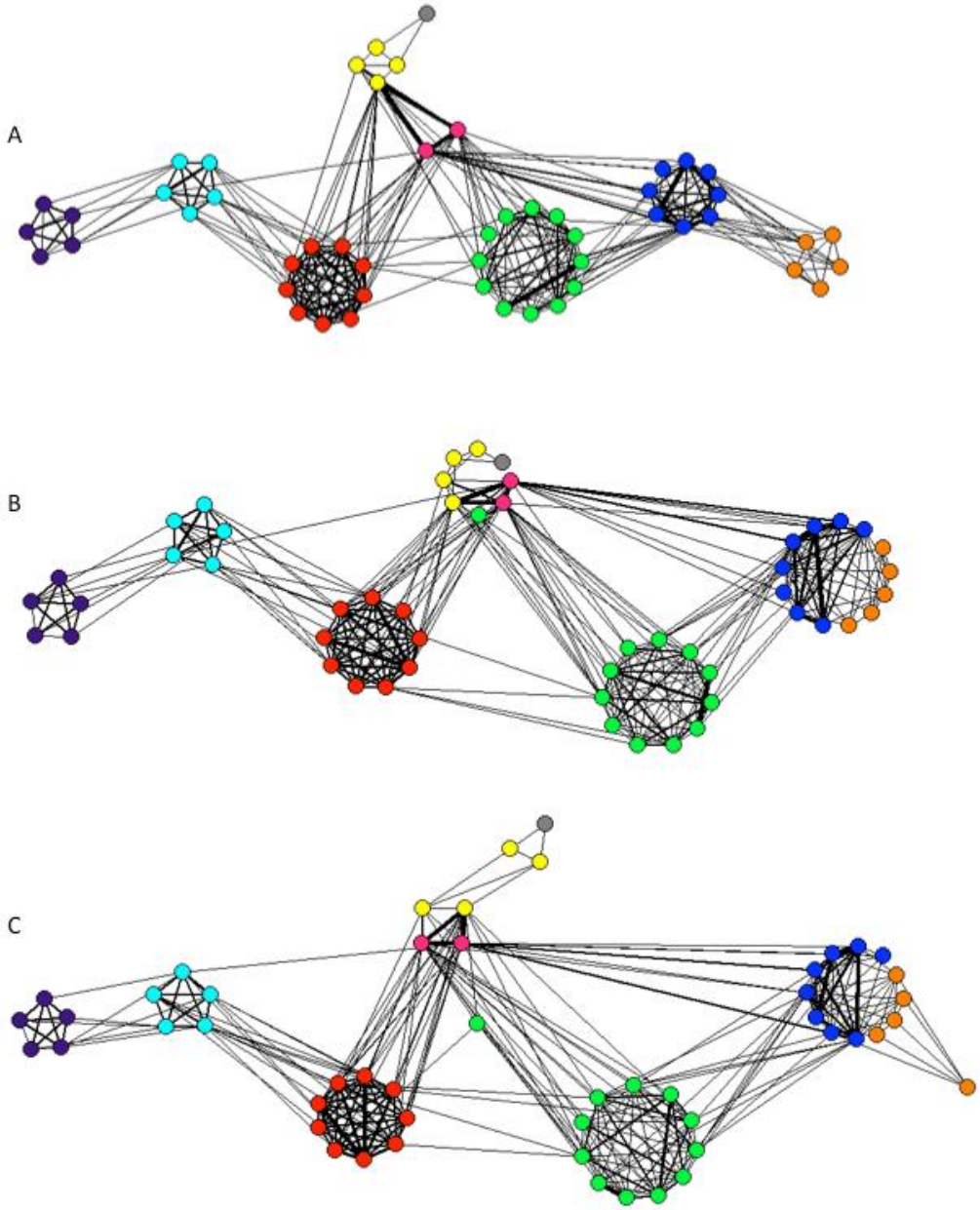


Figure 2.4 Network diagrams of sampled badgers (n=51) divided according to the method of community detection. Communities are arranged corresponding to the spatial location of the social group main sett, but the proximity of nodes to each other is of no relevance. Line thickness is proportional to interaction frequency and nodes are coloured according to the coloured marker in the bait. A = The nine communities identified using the bait marking approach, B = The six communities identified using the fully optimised unrestricted network approach, C = The nine communities identified using the partially optimised restricted network approach.

Comparing methods of community detection

The fully optimised unrestricted social network approach identified six significantly more distinct communities, compared to the nine social groups detected by the bait marking method (mean modularity using bait marking method = 0.636 [95% CI 0.634, 0.638], Figures 2.4 and 2.5). The communities identified using the partially optimised restricted social network approach using nine subdivisions were also more distinct than those identified using the bait marking method (mean modularity of nine network method = 0.731 [0.729, 0.732], Figures 2.4 and 2.5). Similar results were also found in the log and binary networks (Figure 2.5). However, greater variation in modularity estimates led to the difference between the bait marking and social network approaches being reduced, and more variation in the number of communities identified (Figure 2.5, Table 2.3).

The influence of individuals on community structure

Sex was found to significantly influence individual contribution to community structure. Males were found to increase how distinct the communities were from one another (average increase in modularity = 0.004 [95% CI 0.002, 0.007]), whereas females were found to reduce the level of distinction (average increase in modularity = -0.004 [-0.007, -0.002]). Age was also found to be a significant contributor, with sub-adults increasing how distinct the communities were (average increase in modularity = 0.003 [0.0003, 0.0058]), and adults reducing this distinction (average decrease in modularity = -0.003 [-0.0058, -0.0003]). Twenty-one of the fifty-one badgers collared tested positive for bTB. However, infection status was not found to influence the community structure of the population (TB+ average decrease in mod = -0.001 [-0.004, 0.002]; TB- average increase in mod = 0.001 [-0.002, 0.004]).

The implications of method choice

The same results were found when repeating the analysis from Weber *et al.* 2013a using both the original bait marking approach and the fully optimised unrestricted network method of community detection (Figure 2.6, Table S2.1, Table S2.2, Table S2.3). TB test-positive badgers were found to have lower within-group degree and closeness in autumn and winter, higher between-group flow-betweenness in summer and winter, and lower within-group flow-

betweenness in autumn (Figure 2.6, Table S2.1, Table S2.2, Table S2.3). This means that TB test-positive individuals are more influential in disease spread among groups than within groups in these months. However, in addition to this, the network approach also revealed TB test-positive badgers to have higher between-group degree and flow-betweenness in the spring, lower within-group flow-betweenness in the winter, and higher within-group flow-betweenness in the summer (Figure 2.6, Table S2.2, Table S2.3).

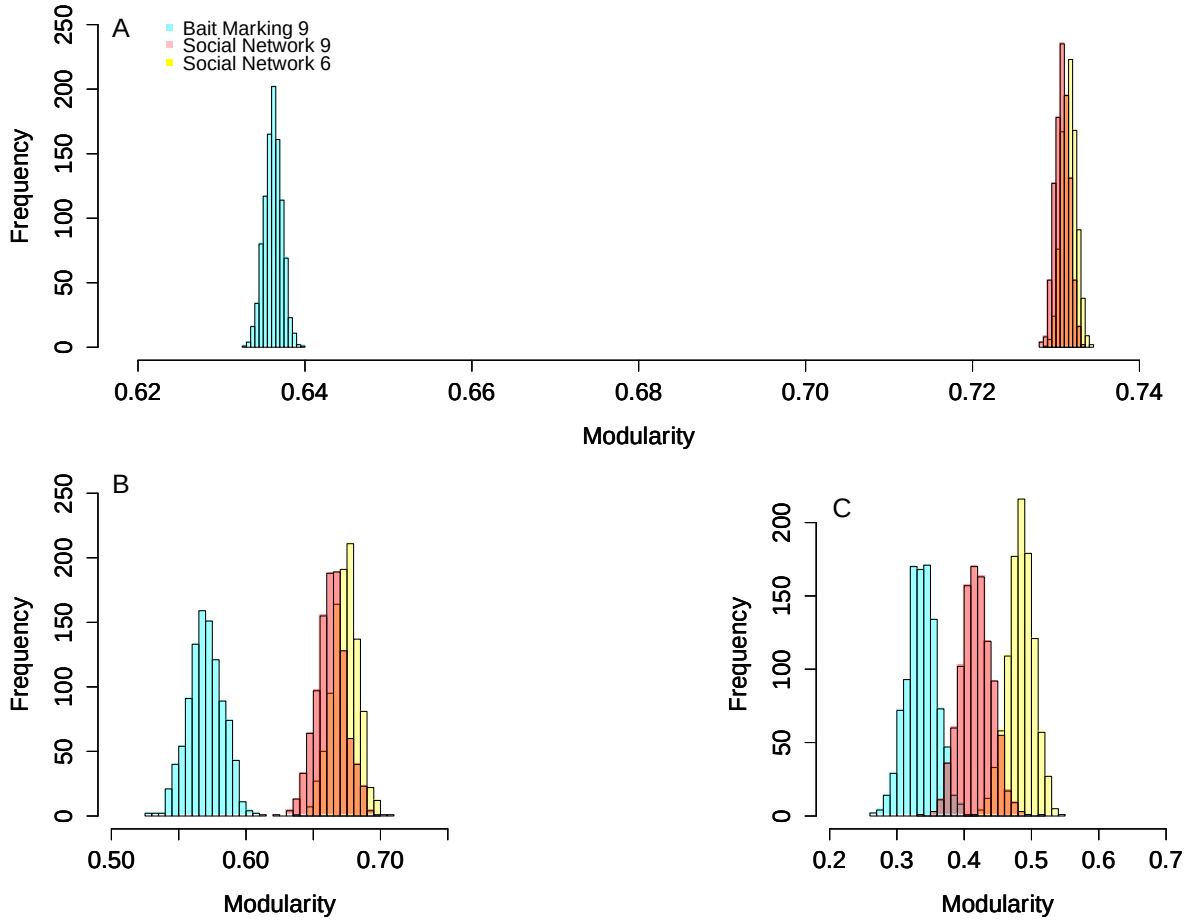


Figure 2.5 Modularity estimates for the network divided into nine communities identified using the bait marking approach (blue), the fully optimised unrestricted network approach into six communities (yellow), and the partially optimised restricted network approach using nine communities (pink). Estimates were determined through bootstrapping badger social networks 1000 times with replacement. Modularity was calculated for each iteration using the multi-level community method in the R package 'igraph' (Csardi & Nepusz 2006) . Estimates for the fully weighted frequency network (A), log(frequency+1) network (B), and binary network (C) are shown.

Table 2.3 Number of communities identified using the fully optimised unrestricted network approach for the fully weighted, log(frequency+1) and binary networks. Estimates were determined through bootstrapping badger social networks 1000 times with replacement using the multi-level community method in the R package 'igraph' (Csardi & Nepusz 2006). The number of communities identified was extracted with each iteration.

Network Type	Number of Communities Detected	Frequency
Fully Weighted	6	100%
Log(Frequency + 1) Weighted	4	0.1%
	5	3.8%
	6	95.9%
	7	0.2%
Binary Weighted	3	1.8%
	4	31.1%
	5	61.2%
	6	5.7%
	7	0.2%

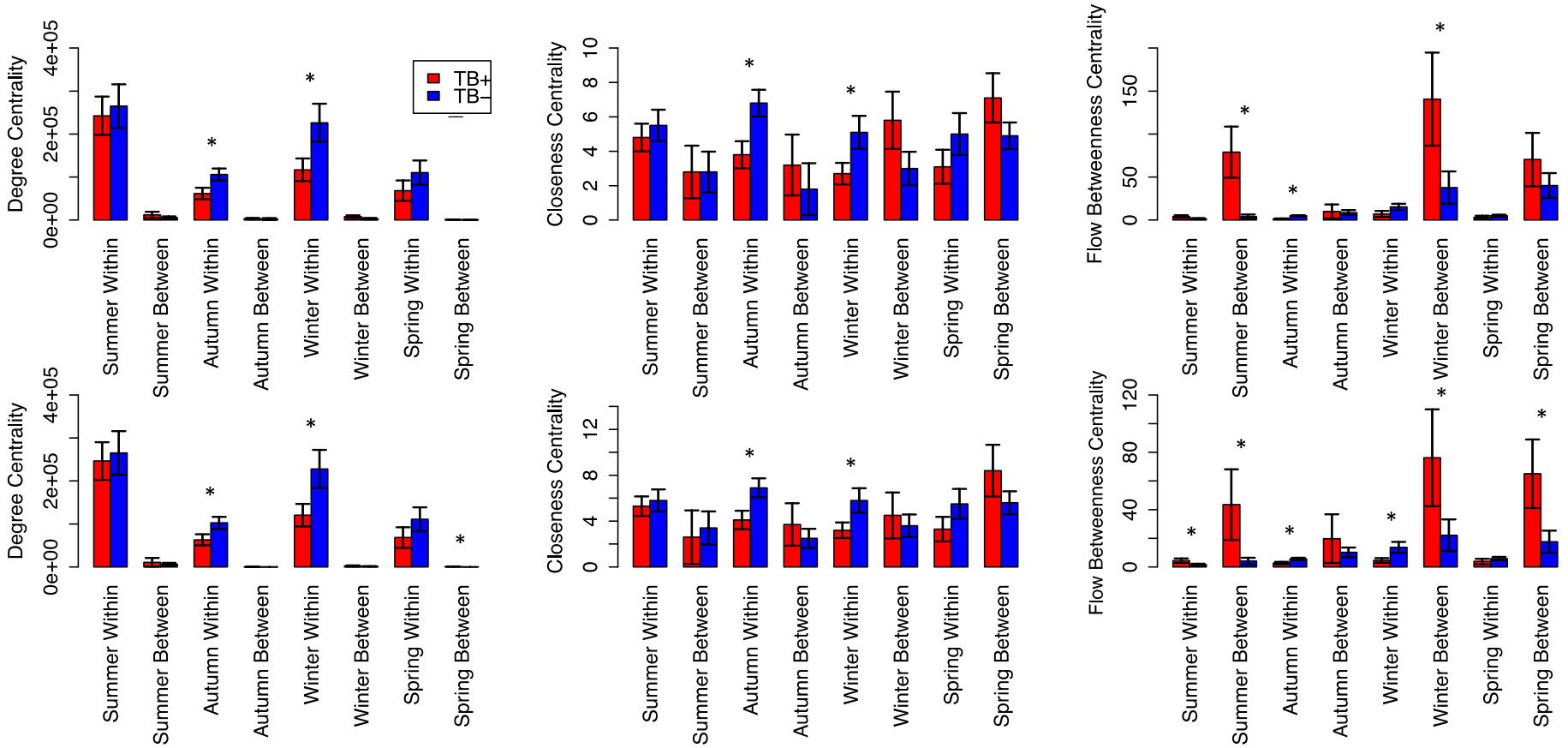


Figure 2.6 Reanalysis of network metrics of TB test-positive and TB test-negative badgers for within- and among-group contacts from Weber *et al.* 2013a. Results using the bait marking method (top panel), and fully optimised unrestricted social network method of community detection (lower panel) are shown. The difference between individuals was tested using node level t-tests in UCINET (Borgatti *et al.* 2002) where TB test outcome was permuted among nodes in 10,000 randomisation trials. Values are means +/- standard error. Significant results are signified with a '*'.

2.5 Discussion

This study aimed to quantitatively determine the community structure of a well-studied badger population. Badgers reside in highly structured communities with distinct groups. Individuals vary in their contribution to this structure, with sex and age found to be influential factors. However, TB test-positive individuals do not influence the community structure of the population, despite being influential for flow among groups. When comparing the indirect bait marking method and the direct unrestricted social network method of community detection, the network method suggests that badgers reside in fewer, more distinct groups than was previously thought. Reanalysing a study that used the bait marking method using this new community structure, gives results that support the original analysis (Weber *et al.* 2013a). However, an improvement in power and reduction in error led to additional findings that suggest TB test-positive badgers may be more influential for among-group disease transmission than was previously thought.

The community structure identified differed depending on the method of detection used, with the unrestricted social network approach identifying six significantly more distinct social groups than the nine originally identified from the bait marking method. These highly distinct communities are likely to inhibit the spread of TB, slowing the transmission of disease through the population through reducing the probability that infection will spread to another group (Liu, Lai & Ye 2003; Altizer *et al.* 2003; Cross *et al.* 2004; Wu & Liu 2008; Jones & Salathe 2010). This finding can help explain why badger social structure is thought to restrict the spread of disease through the population (Delahay *et al.* 2000b). The difference between these two outputs could be explained by the social network approach utilising data of a much finer temporal resolution than the bait marking data; similar differences in data have been found to influence the networks detected in studies of yellow necked mice (*Apodemus flavicollis)* (Perkins *et al.* 2009).

The bait marking approach identifies nine social groups. This gives a less distinct community structure than both the fully optimised unrestricted social network method, and the social network method with optimisation restricted to nine groups. In addition, nine groups are not identified when analysing networks

of different weight, despite the reduction in certainty in which the community structure could be identified, and the greater variation in the number of communities detected. This is due to rare among-group contacts being given equal importance to frequent within-group contacts in the log and binary networks, reducing the distinction between social groups. This is further evidence suggesting that weighted networks should be used in network analysis wherever possible (Farine 2014). Given that the social network approach identifies communities based on contact rates, and differential contact rates significantly influence disease spread (Danon *et al.* 2012), I regard the social network approach to be more representative of badger community structure in the context of disease. Therefore, the use of the weighted, fully optimised unrestricted social network method of community detection may be more appropriate for the study of disease transmission.

When examining the differences between the structures identified by the bait marking and social network method, two sets of groups from the nine merged to make six. As the bait marking data shows little historical affiliation between these merged groups, this suggests that even when using multiple years of bait marking data the bait marking approach may fail to detect the nuances in social community structure detected by the social method. As the communities identified using bait marking are likely to have some biological relevance, for example indicating shared space use for territoriality purposes (Kruuk, Gorman & Leitch 1984), this would be consistent with previous studies that have found shared space use to be a poor indicator of contact rates between individuals (Schauber, Storm & Nielson 2007). This is of particular significance when planning badger management strategies. For example, bait marking is a method commonly used by environmental consultants to determine the level of disturbance building projects may have on resident badgers (Delahay *et al.* 2000a). However if this method fails to detect the social structure of the population, it risks underestimating the level of disturbance. From a disease perspective, the bait marking method has been used in numerous studies concerning badger social organisation (e.g. Christian 1994; Rogers *et al.* 1998; Vicente *et al.* 2007; Woodroffe *et al.* 2009). The collection of badger contact data during these studies to enable the social network method of community

detection to be performed may have improved their relevance to disease dynamics.

Age was found to significantly influence the community structure of the network, with sub-adult badgers increasing how isolated the communities are. This suggests that sub-adults have fewer among-group contacts than adults. This is consistent with evidence suggesting that extra-group paternity accounts for 50% of mating events (Carpenter *et al.* 2005), and older badgers are more likely to move social groups (Rogers *et al.* 1998). Sex was also found to influence community structure, with males increasing how distinct the communities are. This suggests that, compared to females, males interact with members of their own community more frequently than other groups. This finding is supported by evidence that suggests females visit other main setts more frequently (Christian 1994), and males have higher levels of within-group contact at certain times of year (Reed 2011). However as the latter of these two studies relied upon the bait marking approach and evidence is clearly contradictory (Rogers *et al.* 1998; Böhm *et al.* 2008), further investigation of sex differences in among-group contact rates determined using social network methods would help clarify this. Disease status did not influence community structure. This suggests that although high betweenness scores imply TB test-positive individuals are important in linking social groups, they do not significantly contribute to the community structure of the network. This further supports the idea that TB test-positive badgers are “spread-capacitors” of infection due to their role in regulating flow through the network, as opposed to “super-spreaders” that have a disproportionate amount of contact with other individuals (Weber *et al.* 2013a). These distinctive network positions are important for disease dynamics (Corner *et al.* 2003).

Using the social network approach to reanalyse an existing study revealed that TB test-positive badgers have higher within- and among-group flow-betweenness scores than was detected in the original study. As high betweenness scores contribute towards the rapid spread of infection in many systems, including TB in brushtail possums (*Trichosurus vulpecula*) (Corner *et al.* 2003), foot and mouth at pivotal livestock markets (Ortiz-Perlaez *et al.* 2006), and HIV in human populations (Bell, Atkinson & Carlson 1999), TB test-positive

individuals may be occupying even more influential network positions for disease spread than was estimated by the original study. However the cause of these behavioural differences between TB test-positive and TB test-negative badgers cannot be inferred. Although some infections are known to influence host behaviour (Berdoy, Webster & Macdonald 2000), it is unknown if the distinctive network positions that TB test-positive badgers occupy lead to the acquisition of infection as opposed to infection causing this behaviour to occur.

The use of the bait marking method may have caused additional noise in previous studies, such as those that relied upon bait marking to determine how badger movement between groups affects disease dynamics (Rogers *et al.* 1998; Vicente *et al.* 2007). Although my findings showed each method to yield only small qualitative differences in results, given the importance of differential contact rates for disease dynamics (Wey *et al.* 2008), the use of the social network approach when studying disease transmission may be the more appropriate method for future research. However, the extra cost in acquiring network data should be evaluated against the gain in power.

Given how influential community structure is for disease transmission (Altizer *et al.* 2003; Cross *et al.* 2004; Wu & Liu 2008; Jones & Salathe 2010), the ability to effectively detect the social structure of a population to fully understand disease dynamics is clear. Through the use of social network analysis, this study suggests that badgers may be functioning in fewer, more distinct social groups than was previously assumed under bait marking methods, likely inhibiting disease transmission to the entire population. Use of this method has increased the sensitivity of analysis to detect results that would otherwise be overlooked. Therefore, the use of network methods of community detection may be more appropriate for the study of disease transmission.

2.6 Supplementary Information

Table S2.1 Reanalysis of Weber *et al.* 2013a's closeness centrality of TB test-positive and TB test-negative badgers for within- and among-group contacts identified using different methods of community detection. Results using the community structure identified by the bait marking method (Bait Marking) and fully optimised unrestricted social network method of community detection (Network) are shown. The difference between individuals was tested using node level t-tests in UCINET (Borgatti *et al.* 2002) where TB test result was permuted among nodes in 10,000 randomisation trials. Means (standard deviation) and P values are shown. Significant values are in bold.

Season	N	Method	Within-Group			Among-Group		
			TB +	TB -	P	TB +	TB -	P
Summer	39	Network	5.3 (3.7)	5.8 (4.8)	0.34	2.6 (7.4)	3.4 (6.1)	0.45
		Bait Marking	4.8 (3.5)	5.5 (4.6)	0.32	2.8 (5.9)	2.8 (5.4)	0.50
Autumn	44	Network	**4.1 (3.6)**	**6.9 (4.1)**	**0.01**	3.7 (3.7)	2.5 (2.5)	0.27
		Bait Marking	**3.8 (3.5)**	**6.8 (3.8)**	**0.01**	3.2 (5.3)	1.8 (5.0)	0.32
Winter	37	Network	**3.2 (2.7)**	**5.8 (5.0)**	**0.04**	4.5 (4.9)	3.6 (3.5)	0.38
		Bait Marking	**2.7 (2.5)**	**5.1 (4.5)**	**0.03**	5.8 (5.0)	3.0 (4.1)	0.09
Spring	33	Network	3.3 (4.0)	5.5 (5.7)	0.12	8.4 (4.5)	5.6 (3.6)	0.13
		Bait Marking	3.1 (3.7)	5.0 (5.3)	0.13	7.1 (3.2)	4.9 (3.2)	0.12

Table S2.2 Reanalysis of Weber *et al.* 2013a's degree centrality of TB test-positive and TB test-negative badgers for within- and among-group contacts identified using different methods of community detection. Results using the community structure identified by the bait marking method (Bait Marking) and fully optimised unrestricted social network method of community detection (Network) are shown. The difference between individuals was tested using node level t-tests in UCINET (Borgatti *et al.* 2002) where TB test result was permuted among nodes in 10,000 randomisation trials. Means (standard deviation) and P values are shown. Significant values are in bold.

Season	N	Method	Within-Group			Among-Group		
			TB +	TB -	P	TB +	TB -	P
Summer	39	Network	246366 (191349)	265247 (253516)	0.40	10754 (31862)	6654 (12603)	0.36
		Bait Marking	242477 (194255)	265146 (253620)	0.38	12095 (27246)	5823 (11853)	0.23
Autumn	44	Network	**63162 (57938)**	**102730 (68955)**	**0.03**	367 (475)	181 (249)	0.19
		Bait Marking	**61780 (58975)**	**105955 (68491)**	**0.02**	3234 (5391)	2452 (7143)	0.33
Winter	37	Network	**120482 (104594)**	**228035 (206520)**	**0.03**	2280 (3388)	1231 (2131)	0.18
		Bait Marking	**116892 (105663)**	**226236 (207380)**	**0.03**	7901 (8379)	3088 (7906)	0.09
Spring	33	Network	68373 (89341)	110761 (121258)	0.15	**930 (724)**	**320 (323)**	**0.04**
		Bait Marking	68289 (89322)	110543 (121237)	0.15	978 (655)	489 (646)	0.10

Table S2.3 Reanalysis of Weber *et al.* 2013a's flow-betweenness centrality of TB test-positive and TB test-negative badgers for within- and among-group contacts identified using different methods of community detection. Results using the community structure identified by the bait marking method (Bait Marking) and fully optimised unrestricted social network method of community detection (Network) are shown. The difference between individuals was tested using node level t-tests in UCINET (Borgatti *et al.* 2002) where TB test result was permuted among nodes in 10,000 randomisation trials. Means (standard deviation) and P values are shown. Significant values are in bold.

Season	N	Method	Within-Group			Among-Group		
			TB +	TB -	P	TB +	TB -	P
Summer	39	Network	**4.4 (6.6)**	**1.6 (3.3)**	**0.04**	**43.5 (77.8)**	**4.2 (9.2)**	**0.01**
		Bait Marking	4.2 (6.7)	1.6 (3.3)	0.06	**79.0 (115.1)**	**4.1 (10.3)**	**0.002**
Autumn	44	Network	**2.7 (3.8)**	**5.6 (4.1)**	**0.01**	19.7 (34.0)	10.2 (10.2)	0.31
		Bait Marking	**1.5 (2.0)**	**5.0 (3.5)**	**<0.001**	9.9 (24.4)	8.7 (9.8)	0.45
Winter	37	Network	**4.6 (6.5)**	**13.7 (17.9)**	**0.03**	**76.2 (82.6)**	**22.1 (40.0)**	**0.05**
		Bait Marking	7.1 (14.0)	15.4 (16.7)	0.06	**140.6 (162.2)**	**37.8 (79.8)**	**0.03**
Spring	33	Network	3.9 (6.9)	5.8 (5.4)	0.20	**65.0 (47.8)**	**17.6 (27.6)**	**0.03**
		Bait Marking	3.3 (6.8)	5.0 (5.0)	0.23	70.4 (69.3)	40.2 (59.1)	0.18

Chapter 3
Relatedness and Badger Social Contacts

3.1 Abstract

1. In social groups that consist of related individuals, relatives have been observed to exhibit higher contact rates with one another compared to non-relatives. The European badger (*Meles meles*) is a facultatively social mammal that, in parts of its range, lives in highly related social groups. However, whether related badgers preferentially associate with one another is currently unknown.
2. This study aimed to determine whether badgers preferentially associate with their kin. Similar assortative mixing has been documented in other species; therefore, within-groups I expected related badgers to spend more time together. However, given that extra-group mating is thought to reduce inbreeding in badger populations, I expected extra-group contact to occur between less related individuals. Badgers are an important reservoir of bovine tuberculosis. Therefore, I also discuss the possible implications of these patterns of association for disease transmission.
3. By using empirically derived contact and relatedness data from a wild badger population, I found that although related individuals spend more time together, this is a product of shared space use amongst relatives, and not evidence for the preferential association of kin. However among groups, badgers were found to associate preferentially with less related individuals.
4. These findings suggest that something other than kinship, such as resource distribution, may be better able to explain the social cohesion of badgers at high densities. Given that extra-group contact was more likely to occur between less related individuals, this may suggest that extra-group contact may provide a mechanism for inbreeding avoidance in the population. Therefore, inbreeding avoidance may facilitate disease spread among badger groups.

3.2 Introduction

The study of social networks can allow new insights to be gained into the associations between individuals, identifying clusters of individuals that are more connected to each other than to the rest of the population (Newman 2002; Palla *et al.* 2005; Oliveira & Gama 2012). Living in groups can offer benefits to individuals, including foraging advantages (Gompper 1983), predator defence (Molvar & Bowyer 2006), higher quality territories (Mosser & Packer 2009), and thermoregulation (Arnold 1990). Groups can also provide protection against disease spread, with members less likely to acquire infection from the wider population (Jones & Salathe 2010). Therefore, behaviours that influence clustering within the population can be highly important for disease transmission.

Within a population, social groups can arise when individuals preferentially associate with others. This commonly occurs between individuals that are similar, for example those that are of a similar age (Wey & Blumstein 2010), or personality type (Massen & Koski 2014). Some social groups consist of related individuals, often as a product of natal philopatry where offspring are retained within the group and do not disperse (Greenwood 1980). This can lead to kin mixing assortatively, with related individuals exhibiting higher contact rates than non-relatives. This has been observed in many species including sperm whales (*Physeter macrocephalus*) (Gero *et al.* 2008), salmon (*Salmo salar*) (Griffiths & Armstrong 2002), elephants (*Loxodonta africana*) (Archie *et al.* 2006), dolphins (genus *Tursiops*) (Wiszniewski *et al.* 2010), and macaques (*Macaca mulatta*) (Widdig *et al.* 2001).

The European badger (*Meles meles*) is a facultatively social mammal that is group-living at medium to high densities (Tuyttens *et al.* 2000). Individuals within these mixed-sex groups defend communal territories which can remain stable over many years (Kruuk 1978, **chapter 2**). At night when they are active, badgers are thought to be fairly solitary, but do interact with others both from their own group and also neighbouring groups, for example when foraging, mating or during aggressive encounters (Roper 2010). Interactions can also occur between individuals when they are resting, especially when individuals share nest chambers within the underground sett in which they sleep (Roper *et*

al. 2001). These contacts can have important consequences for the transmission of bovine tuberculosis (bTB), for which badgers are an important reservoir in the UK.

Bovine tuberculosis is a chronic disease of cattle that is caused by the bacterium *Mycobacterium bovis.* Historically, this disease has been managed in developed countries through cattle test-and-slaughter programmes and restrictions upon trade and animal movements (Krebs *et al.* 1997; Michel *et al.* 2009). However, these management practices are undermined in the UK by the presence of badgers as a wildlife reservoir of disease. Badger social structure and movement behaviour has been shown to influence bTB disease dynamics in badgers and in cattle. For example, increased movement of badgers between social groups has been associated with an increase in disease incidence within the badger population (Rogers *et al.* 1998; Vicente *et al.* 2007). The changes in badger spatial behaviour following culling, such as increased dispersal rates, larger range sizes, and greater social group overlap (Woodroffe *et al.* 2006; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011), have also been linked to an increase in bTB prevalence in cattle (Donnelly *et al.* 2003, 2006; Woodroffe *et al.* 2006). Therefore identifying the factors that influence contact rates between individuals, both within- and among-social groups, may help to understand the dynamics of this disease.

Within badger social groups, natal philopatry and low permanent dispersal rates lead to high levels of relatedness (Da Silva *et al.* 1994; Carpenter *et al.* 2005). Additionally, in an attempt to avoid inbreeding (Carpenter *et al.* 2005), high levels of extra-group mating results in relatedness being spatially clustered between neighbouring social groups (Da Silva *et al.* 1994; Carpenter *et al.* 2005; Dugdale *et al.* 2008). It has previously been suggested that the social cohesion of badgers is maintained by these high levels of relatedness (Dugdale *et al.* 2008). This hypothesis is consistent with findings on other social species that show relatives to have higher contact rates if given the opportunity (e.g. Widdig *et al.* 2001; Griffiths & Armstrong 2002; Archie *et al.* 2006; Gero *et al.* 2008; Wiszniewski *et al.* 2010). However, to date there has been no direct test of whether badgers mix assortatively with their relatives.

This study aims to determine whether badgers interact assortatively with kin, and discuss what implications this might have for disease transmission. To achieve this, the duration of time individuals spend together and their relatedness will be compared. Spatial proximity can often predict the social proximity of individuals (Sih, Hanser & McHugh 2009). Therefore, the spatial proximity of individuals to one another will also be accounted for. This will enable individuals that spend more time together, beyond what can be expected given their spatial proximity, to be identified. Nocturnal and diurnal interactions will be analysed separately to determine if badgers associate differently with relatives when they are active during the night compared to when they are resting during the day. Within- and among-group contacts will also be analysed separately. Given that the preferential association of kin has been observed in other species, I expect to find that within-groups related badgers spend more time together than unrelated individuals. However, among-group contact is expected to be negatively correlated with relatedness to reflect the high levels of extra-group mating that occurs to reduce inbreeding within the population (Carpenter *et al.* 2005; Annavi *et al.* 2014). These findings will give insight into the drivers of social interactions amongst badgers, and improve the understanding of TB transmission.

3.3 Methods

Study site

Woodchester Park (N51°42' 34", W2°16' 26") is situated on the Cotswold limestone escarpment in Gloucestershire, South West England. The core study area of 7km^2 comprises of mixed woodland, pasture and arable farmland, and has a resident high-density badger population that is the subject of a long-term capture-mark-recapture study (Delahay *et al.* 2000b).

Badger sampling

Trapping events at Woodchester Park are carried out approximately 4 times a year using methods described in Delahay *et al*. 2006a. Briefly, badgers were caught using steel mesh cage traps that are baited with peanuts. Badgers were then anaesthetised using an intramuscular administration of two parts butorphanol tartrate (Torbugesic®, Wyeth, Ontario, Canada), two parts ketamine hydrochloride (Ketaset®, Wyeth, Ontario, Canada) and one part

medetomidine (Domitor®, Orion Corporation, Espoo, Finland) (De Leeuw *et al.* 2004). For each individual, the age, sex and capture location was recorded, and a hair sample taken and stored in 80% ethanol. Fifty-one badgers that were captured as part of the long-term study were collared with proximity loggers (Sirtrack, New Zealand) over 11 days between May and October 2009. The age, sex and capture locations of these individuals are summarised in Table 3.1 and Figure 2.1.

Table 3.1 The demographic classes of the 40 badgers analysed in this study. Numbers given in brackets indicate the total number of badgers collared (51). Functional group membership was determined using modularity analysis, and the advertised group in which each badger was caught is also given.

Functional social group	Advertised spatial group	Sex		Age	
		Male	Female	Adult	Sub-adult
Group 1	West	3 (3)	1 (2)	2 (3)	2 (2)
Group 2	Larch	3 (3)	2 (2)	3 (3)	2 (2)
Group 3	Beech	4 (4)	5 (5)	4 (4)	5 (5)
Group 4	Cedar	1 (2)	2 (2)	2 (3)	1 (1)
	Breakheart	0 (0)	1 (1)	1 (1)	0 (0)
	Septic Tank	1 (1)	1 (1)	0 (0)	2 (2)
Group 5	Top/Yew	3 (6)	3 (6)	3 (8)	3 (4)
Group 6	Wych Elm	5 (5)	2 (3)	4 (4)	3 (4)
	Kennel	0 (0)	3(5)	1 (1)	2 (4)

Contact events

Proximity collars recorded badger contact data between May 2009 and May 2010. These collars contained an Ultra High Frequency (UHF) transceiver that broadcasts a unique ID code whilst simultaneously ‘listening’ for those of others (Drewe *et al.* 2012). When loggers came within a defined distance of one another, a contact was initiated until a signal could no longer be detected, details of which were stored on its internal memory (Drewe *et al.* 2012). The loggers were programmed to record interactions when within 0.64+/- 0.04m of one another (UHF range 34-48). This enabled interactions that occurred at close distances (e.g. fighting, grooming and mating) to be recorded, although different types of interaction cannot be differentiated in the data. Of the 51 badgers collared, 11 had collars that were not retrieved. This was due to either the collar being dropped underground, or the badger not being recaptured at

the end of the study. However, contact data were downloaded whenever badgers were recaptured throughout the year. All contacts that occurred during trapping operations and the subsequent 12 hours were omitted from the analyses. Of the 51 badgers collared, sufficient contact data could be collected for 44 individuals.

Proximity loggers have the tendency to record extended interactions as a series of shorter contacts (Drewe *et al.* 2012). Therefore, to improve the accuracy of the interaction data the protocols suggested by Drewe *et al.* 2012 were followed; all interactions that were recorded within 1.5 minutes between the same pair of loggers were amalgamated, and any additional 1-second interactions were removed. These contacts were then amalgamated into a matrix ready to be analysed using an R script (Reed 2011). To account for temporal differences in badger behaviour, for example resting during the day and foraging at night, separate matrices were created for the complete network (all contact events), the nocturnal network (interactions that occurred when badgers were active between 8pm-6am), and the diurnal network (interactions that occurred when badgers were resting between 6am-8pm). Given that the frequency and duration of contact events recorded by these proximity loggers are known to be highly correlated (Reed 2011), but contact duration deemed slightly more accurate (Drewe *et al.* 2012), interactions for this analysis were weighted by contact duration.

Relatedness Estimates

Hair samples taken during badger sampling were submitted for genotyping and DNA extraction. Of the 51 badgers collared, 44 genotypes could be derived using 22 microsatellite markers, each with 4-7 alleles. The MicroDrop Programme (Wang 2012) was used to impute missing data in the microsatellite dataset, which adjusts for allelic dropout in the genotypes (Miller, Joyce & Waits 2002). Deviations from Hardy-Weinberg equilibrium for each of the 22 microsatellite markers were tested on the MicroDrop-corrected dataset using the hwtest function in the ‘adegenet’ package (Jombart *et al.* 2008); none was identified. The Bartlett test of homogeneity was also used to confirm homogeneity of variance among loci, also using the ‘adegenet’ package (Jombart *et al.* 2008). The R package ‘Demerelate’ (v 0.8-1) (Kraemer &

Gerlach 2013) was used to estimate relatedness, using the Queller and Goodnight r_{xy} relatedness estimator (Queller & Goodnight 1989). This provides an unbiased estimate of relatedness based on the population allele frequencies, ranging from -1 to 1 with negative and positive values indicating lower- and greater-than-average relatedness, respectively (Grear *et al.* 2010). A matrix of relatedness for each pair of individuals was created.

Index of spatial proximity

In order to determine if the spatial clustering of individuals within the population could better explain badger contact rates compared to relatedness, an index of spatial proximity was calculated. This index is based on the number of times each pair of badgers was caught within the same geographical area between 2005 and 2009. For this analysis, these geographical areas were taken as the advertised badger group territories, which are determined using bait marking data. To calculate the index of spatial proximity, bait marking and capture location data collected between 2005 and 2009 were used.

Bait marking data were collected using methods described in detail in Delahay *et al.* 2000a. Briefly, the bait marking method utilised badger latrines, which are shallow pits in which badger excretory products are deposited (Delahay *et al.* 2000a), and are typically used to demarcate the territory boundaries of badger social groups (Kruuk 1978). Each of these territories usually has an associated main sett which is permanently occupied and used by all members of a group (Roper 1992). Baits containing peanuts, syrup and small coloured indigestible plastic pellets were placed at these setts (Figure 2.2), with a differently coloured or shaped pellet used at each. The presence of pellets in badger faeces was then recorded during surveys of the study area for badger latrines. This allowed each latrine to be attributed to a specific sett in each year. Bait return data were used alongside field observations, such as well-used badger paths between adjacent territories, to infer territory boundaries. The territories identified are thought to give information on the configuration of badger social groups within the population (Delahay *et al.* 2000a). In the population studied, bait marking is carried out each spring when latrine use is at its highest (Kruuk 1978), and vegetation is low. This makes latrines easier to find, and so territory boundaries

easier to identify. At every capture event, the advertised territory that each badger was caught in was recorded.

The advertised territories that the 51 badgers collared for this study were caught in between 2005-2009 was used to calculate the index of shared spatial proximity using the following equation:

$$\frac{(\text{No. times A caught in same advertised territory as B}) + 1}{(\text{No. times A caught in same advertised territory as B}) + (\text{No. times A caught without B}) + (\text{No. times B caught without A}) + 1}$$

This equation uses the number of times each pair of badgers (e.g. Badger A and Badger B) was caught within the same advertised territory between 2005 and 2009. The total number of times each individual was captured over this same time period was also included. In addition, '+1' was added to the numerator and denominator to ensure individuals that were caught frequently, but never together, could be distinguished from those that were caught infrequently. This equation gave a value ranging between 0 and 1 for each pair of individuals, with 0 indicating that badgers were never caught within the same advertised territory, and 1 indicating that the badgers were always caught within the same advertised territory. These values were then amalgamated into a matrix.

Within- and among-group contacts

For the purpose of differentiating within- and among-group contacts, social network analysis was used to identify the community structure of the population (Newman 2006, **chapter 2**). These functional communities were identified by calculating network modularity using the R package 'igraph' (Csardi & Nepusz 2006). This metric identifies divisions in the social network, and is formally defined as the fraction of within-group edges in the observed network minus the expected fraction of within-group edges in a randomised null model (Newman 2006). This null model is based on the observed network graph, but rearranges the edges randomly with no regard to community structure (Newman 2006).

Modularity (Q) is calculated using the following equation:

$$Q = \frac{1}{2m}\sum_{ij}\left[A_{ij} - \frac{k_i k_j}{2m}\right]\delta_{gigj}$$

(Newman 2011)

Where the expected number of edges falling between two vertices (*i* and *j*) is equal to $k_i k_j/2m$, where *k* is the node degree and *m* is the total number of edges in the observed network. The actual number of edges observed between two nodes is equal to A_{ij}. An integer label is given to each node denoting the group it belongs to in the proposed network division (g_i), and δ_{ij} is the Kronecker delta which tests whether the nodes belong to the same group (Newman 2011).

By comparing the observed to the null networks it can be determined whether the number of within-group edges is greater than would be expected by chance (Newman 2006), and gives a Q value that can range from -1/2 to 1. If Q<0 then no community structure is identified, but if Q=1 then highly structured communities have been detected. Q was optimised to identify divisions in the network, and therefore the social groups present (Newman 2006). This was done by first placing each individual in a separate community and calculating the modularity. Neighbouring communities were then joined to produce the largest increase in modularity possible. This process was repeated until the pattern of network division that gave the highest modularity score was found (Clauset *et al.* 2004; Blondel *et al.* 2008; Verdolin *et al.* 2014). All analyses took into account the complete network and edge weight, which was taken as the frequency of interactions, and were carried out using the multi-level community function in the R package 'igraph' (Csardi & Nepusz 2006). The identified communities were then used to create contact matrices for both within- and among-group interactions.

Statistical analysis

To determine if contact rates were higher between related individuals, the contact matrix weighted by contact duration was regressed on the relatedness matrix, whilst taking into account the spatial proximity matrix using ordinary least squares. Given the non-independent nature of network data (Croft *et al.*

2011), the resulting coefficients were tested using the QAP permutation test, using Dekker's "semi-partialing plus" procedure (Dekker, Krackhardt & Snijders 2003, 2007). This was carried out using the function ‘netlm’ in the R package ‘SNA’ (Butts 2014). P values were derived from these permutations to determine if the relationship was significant, with model fit represented by an r^2 value. However, r^2 values are based on the original non-independent regression, and so can only give guidance regarding the relationship between matrices. The contact matrix was then regressed on the relatedness and spatial proximity matrices separately so the explanatory power of each variable in isolation could also be determined. The resulting three r^2 values were then compared to indicate which was the best fitting model. To determine if the correlation between relatedness and social contacts differed with different types of behaviour, the analysis was repeated for the nocturnal, diurnal, within- and among-group networks.

3.4 Results

Of the 51 badgers collared, 11 had insufficient contact and relatedness data for analysis. Therefore 40 individuals were included in the analysis.

Overall, badgers were found to interact significantly more with related individuals than with unrelated individuals (r^2 value = 0.12, $p<0.001$, Table 3.2), but also spent more time with those to which they were spatially close (r^2 = 0.44, $p<0.001$, Table 3.2). When spatial proximity was taken into account, badgers were found to spend no more or less time with relatives than with any other individual (r^2=0.44, p=0.11, Table 3.2, Figure 3.1). Additionally, when comparing the r^2 values of each of these models, model fit was not improved with the addition of the relatedness data, compared to just including spatial proximity in the model (influence of relatedness on within-group contact rates taking into account spatial proximity r^2 value =0.44, relatedness only r^2=0.12, spatial proximity only r^2=0.44, Table 3.2). Similar results were also found for the nocturnal and diurnal networks (Table 3.2).

When analysing within-group contacts only, similar results were found; related individuals did not interact any more or less with relatives than non-relatives when spatial proximity was taken into account (r^2 value = 0.22, p=0.47, Table

3.3). Spatial proximity explained more variation in contact rates, and model fit was not found to increase with the inclusion of relatedness in the model (influence of relatedness on within-group contact rates taking into account spatial proximity r^2 value=0.22, relatedness only r^2=0.06, spatial proximity only r^2=0.22, Table 3.3). However, relatedness was found to significantly influence among-group contact rates, even when spatial proximity was accounted for; related individuals were found to spend significantly less time together (p=0.003, Table 3.3). However, although this effect was significant, both relatedness and spatial proximity could only explain a very small amount of variation in contact rates between individuals (influence of relatedness on among-group contact rates taking into account spatial proximity r^2 =0.008; relatedness only r^2=0.009; spatial proximity only r^2<0.001, Table 3.3).

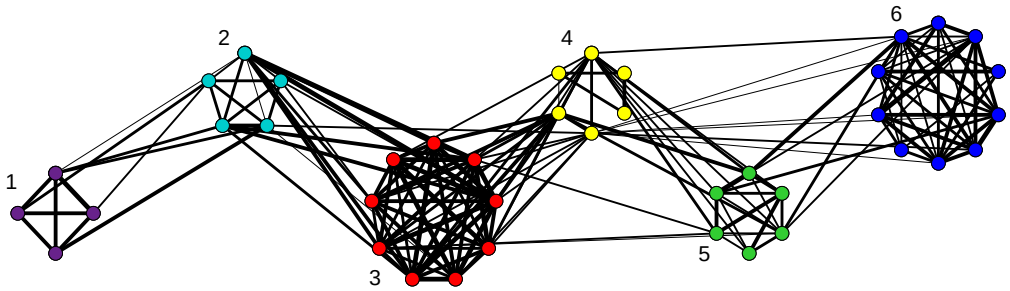


Figure 3.1 Network diagram of sampled badgers (n=40). Position and colour indicate the social groups identified using modularity optimisation. Nodes are arranged corresponding to the spatial location of the social group, but the proximity of nodes to each other is of no relevance. Lines between nodes indicate the presence of an interaction, with line thickness proportional to their relatedness. Numbers indicate the functional social group number.

Table 3.2 Effects of relatedness, spatial proximity and relatedness + spatial proximity on the duration of contacts between individual badgers. Analyses were conducted for the networks of all contacts, nocturnal contacts (8pm-6am), diurnal contacts (6am-8pm). All analysis was calculated using linear regression of network data tested using the QAP permutation test, using Dekker's "semi-partialling plus" procedure in the R package 'sna' (Butts 2014). Estimates are the average increase in contact duration. P values were derived from the permutation tests, however r^2 values are based on the original non-independent regression and so are only given as guidance. Significant results are shown in bold.

	All Contacts			Nocturnal Contacts			Diurnal Contacts		
	Estimate	*P*	R^2	*Estimate*	*P*	R^2	*Estimate*	*P*	R^2
Contact ~ Relatedness	**50352**	**<0.001**	**0.12**	**12558**	**<0.001**	**0.11**	**33450**	**<0.001**	***0.12***
Contact ~ Spatial Proximity	**139199**	**<0.001**	**0.44**	**35176**	**<0.001**	**0.41**	**92242**	**<0.001**	**0.44**
Contact ~ Relatedness + Spatial Proximity	7036	0.11	0.44	1566	0.15	0.41	4771	0.08	0.45

Table 3.3 Effects of relatedness, spatial proximity and relatedness + spatial proximity on the duration of contacts between individual badgers for both within and among-group contacts. Social groups were determined through optimum division of the network using modularity estimates. All analysis was calculated using linear regression of network data tested using the QAP permutation test, using Dekker's "semi-partialling plus" procedure in the R package 'sna' (Butts 2014). Estimates are the average increase in contact duration. P values were derived from the permutation tests, however r^2 values are based on the original non-independent regression and so are only given as guidance. Significant results are shown in bold.

	Within-Group Contacts			Among-Group Contacts		
	Estimate	*P*	R^2	*Estimate*	*P*	R^2
Contact ~ Relatedness	**67312**	**<0.001**	**0.06**	**-2241**	**0.004**	**0.009**
Contact ~ Spatial Proximity	**106041**	**<0.001**	**0.22**	-3556	0.146	<0.001
Contact ~ Relatedness + Spatial Proximity	1956	0.47	0.22	**-2163**	**0.003**	**0.008**

3.5 Discussion

This study aimed to determine if badgers mix assortatively with their kin. In general, related individuals were found to spend more time together, as did individuals that were spatially close. However, when this spatial proximity was accounted for, badgers were not found to interact assortatively with their relatives. Similar patterns were found when badgers were resting during the day, when they were active at night, and when they were interacting with badgers from their own group. This suggests that while badgers may spend more time with relatives, this is only likely to be a product of their spatial proximity, as opposed to relatives preferentially associating with one another. However among groups, related individuals spent significantly less time together compared to non-relatives.

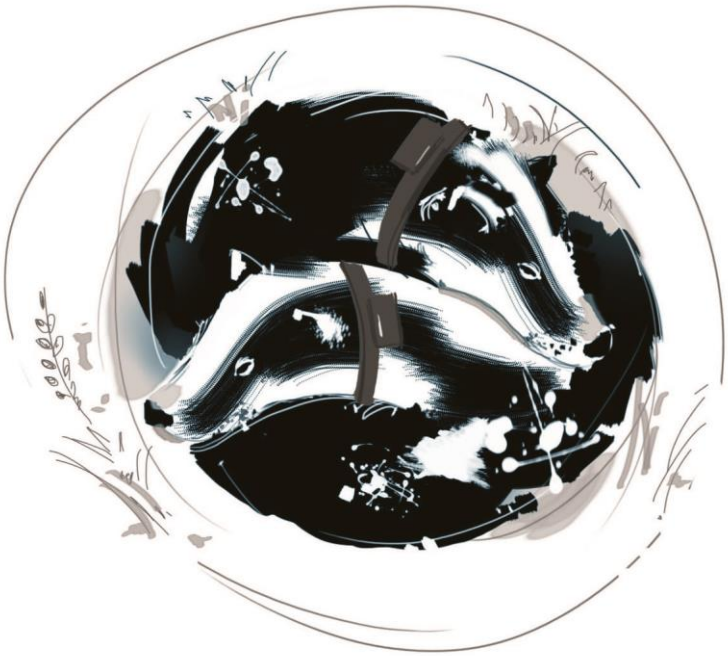
Badgers did not spend any more time with relatives compared to any other individual that they were spatially close to. This was unexpected given that many other social species mix assortatively with their kin (e.g. sperm whales (Gero *et al.* 2008), salmon (Griffiths & Armstrong 2002), and elephants (Archie *et al.* 2006)). However, similar results have been recorded in raccoon (*Procyon lotor*) populations. Like badgers, raccoons are facultatively social, only associate at high densities, and do not mix assortatively with kin (Hirsch *et al.* 2013). It was therefore suggested that kinship might not be a dominant driver of sociality in raccoons (Hirsch *et al.* 2013). Given the similarities, my results might therefore suggest that this is also the case in badgers, with something other than kinship explaining why badgers are social at high densities. This would be consistent with previous studies that have found a lack of kin selective behaviour, such as alloparenting, that are common in other social species where group formation is grounded in familial relationships (Dugdale *et al.* 2008). For example, a study on fossorial mammals found that suitable resource distribution can lead to natal philopatry, and the formation of social groups that often do not engage in cooperative behaviour (Noonan *et al.* 2015). Therefore, my results could give further evidence that the distribution of resources, such as foraging patches or denning locations, may be better able to explain why badgers are social at high densities (Macdonald 1983; Da Silva, Woodroffe & Macdonald 1993; Woodroffe & Macdonald 1993).

Badgers spent more time with less related individuals during extra-group encounters. Given that high levels of extra-group mating have previously been documented in badger populations (Carpenter *et al.* 2005; Dugdale *et al.* 2008), this could indicate that individuals are actively avoiding inbreeding whilst also avoiding the costs of permanent dispersal (Carpenter *et al.* 2005; Dugdale *et al.* 2008). This is supported by a previous study on a different badger population that found extra-group mating increased when within-group relatedness between mothers and candidate fathers was high (Annavi *et al.* 2014). However the use of automated proximity logger technology in this study means that the actual behaviour that occurred when individuals were associating remains unknown. For example, aggressive encounters cannot be differentiated from mating encounters. Therefore, more in-depth analysis to determine if contacts between less related individuals occurred mainly between males and females might help determine if this behaviour is related to mating activity.

These findings offer potential insight into bTB disease dynamics. A recent study has shown that cubs are more likely to acquire infection when close relatives are TB test-positive (Benton *et al.* 2016). This finding could be explained if cubs had higher contact rates with relatives compared to others in the population. If this were the case then if only a proportion of adults were vaccinated in a social group, related cubs would be expected to benefit from some protection given that they could no longer acquire infection via related adults (Benton *et al.* 2016). However, the lack of assortative mixing found amongst related adults in this study indicates that this protection would be lost post emergence. Alternatively, a heritable aspect of infection susceptibility could explain why cubs are more likely to acquire infection when close relatives are infected (Benton *et al.* 2016). This theory would explain both the lack of assortative mixing found between adult relatives in this study, and why cubs are more likely to acquire infection if their relatives are TB test-positive. However, the relationship between genetic variation and TB infection is yet to be determined. In addition, group living can offer protection against disease spread (Jones & Salathe 2010), with the stable badger social structure thought to limit among-group disease transmission (Delahay *et al.* 2000b). However, my results suggest that inbreeding avoidance could be facilitating among-group contact, and therefore disease transmission in badgers. This could reduce the protection

that group living can offer, allowing disease to spread to areas of the population that might otherwise have remained free of infection.

Chapter 4
Badger Sett Use and Ranging Behaviour



4.1 Abstract

1. Behavioural variations in individual space use can have important implications for disease transmission. Therefore, through studying this variation insights can be gained into how disease can spread through a population. European badgers (*Meles meles*) are a wildlife reservoir of bovine tuberculosis (bTB). Badgers are group-living animals, but individuals vary in their home ranging behaviour and their use of dens (setts).
2. This study aimed to determine how patterns of sett use by badgers are related to ranging behaviour. TB test-positive badgers are known to use setts away from the social group's main sett more frequently, and have larger home ranges. Therefore, badgers that frequently use outlying setts were expected to have larger home ranges and spend more time in neighbouring group territories. How these relationships vary with season was also determined, and the implications of these relationships for the spread of bTB discussed.
3. Through using diurnal and nocturnal radio tracking data, I found that the relationship between home ranging behaviour and sett use varies with season; in the autumn, badgers that frequently use outlying setts spend less time in neighbouring social group territories compared to those that reside at the main sett. Conversely, in the winter and spring badgers that use outlying setts more frequently spend more time in neighbouring group territories compared to those at the main sett.
4. These findings suggest that the function of outlier setts may change throughout the year. Badger mating activity peaks in the spring, possibly suggesting that badgers use outlier setts to facilitate extra-group mating at this time. A smaller mating peak also occurs in the autumn, but outlier sett use was not associated with home range overlap. This might suggest that outlier setts serve a different purpose in the autumn. Given that TB test-positive badgers use outlier setts more frequently than TB test-negative badgers, this could indicate that greater home range overlap associated with outlier use in the spring may increase the risk of acquiring and transmitting disease. This could provide the mechanism in which outlier use is associated with TB infection in badgers.

4.2 Introduction

Different species use space in different ways: some are territorial in order to defend mates (Cant, Otali & Mwanguhya 2002); others are nomadic to take advantage of seasonal food abundances (Hillman 1988); and some range further to meet their nutritional needs (Harestad & Bunnell 1979). How space is used while resting can also vary between species, with some showing greater site fidelity than others (Lewis 1995). Within the same species, variation in space use can also be observed, and can be linked to age, sex, or dominance hierarchies. For example, non-breeding female spotted hyenas (*Crocuta crocuta*) of low rank typically range further and spend more time at territory boundaries compared to more dominant individuals (Boydston *et al.* 2003). Similarly, male meerkats (*Suricata suricatta*) are more likely to visit neighbouring territories than female meerkats (Doolan & Macdonald 1996).

These differences in how space is used can have important implications for the acquisition and transmission of disease. For example, territorial African bovids typically have higher nematode loads compared to non-territorial species (Ezenwa 2004). Young adult black-backed and side-striped jackals (*Canis mesomelas* and *Canis adustus*) spend more time in the peripheral areas of the communal range compared to other individuals, leading to elevated among-group contact rates and an increase in rabies transmission (Loveridge & Macdonald 2001). Variation in den sharing rates between captive brushtail possums (*Trichosurus vulpecula*) also leads to some individuals being more socially connected than others, resulting in an increase in bovine tuberculosis transmission (Corner *et al.* 2003). Therefore, through studying variation in space use, insights can be gained into how disease can spread through a population.

At medium to high densities, the European badger (*Meles meles*) is group living (Tuyttens *et al.* 2000). These groups occupy a communal territory, with individuals occupying their own home ranges that overlap with other group members (Kruuk 1978). Group territories can remain stable over many years, and are demarcated by group members using latrines (Kruuk 1978); shallow pits in which excretory products are deposited along with scent marks from faeces, urine, and secretions from the anal, subcaudal and interdigital glands

(Delahay *et al.* 2000a). Although contact rates between these social groups is generally limited (Tuyttens *et al.* 2000), individuals are known to vary in their adherence to these boundaries, with males and older badgers more likely to move between social groups (Rogers *et al.* 1998). In some cases, high levels of among-group contact can result in multiple groups effectively functioning as one (**chapter 2**). This can lead to differences between the group territorial boundaries as "advertised" using latrines and the actual or "functional" boundaries of the group (**chapter 2**).

During the day, badgers sleep in underground burrows known as setts. These setts can be broadly split into two categories: main and outlier. Typically, in medium to high-density populations, main setts are large, have many entrance holes and chambers, and are normally permanently inhabited (Kruuk 1978), whereas outlier setts tend to be smaller, disconnected from the main sett, and less frequently used (Kruuk 1978). Outliers are also typically found nearer to territorial boundaries (Roper 2010). Many theories exist regarding the function of outlier setts, including their role in providing refuges for persecuted subordinates (Kruuk 1989), resting places when foraging away from the main sett (Roper 1992), and ectoparasite avoidance (Roper *et al.* 2001). Individuals are known to vary in their use of these outlier setts, for example females use the main sett more frequently than males during the spring, and adult males generally spend less time at the main sett compared to sub-adult males (Weber *et al.* 2013b).

In the UK, badgers are an important wildlife reservoir of bovine tuberculosis (bTB). This chronic disease of cattle is caused by the bacterium *Mycobacterium bovis*, and globally causes great economic loss through reduced productivity, loss of trade, and compensation pay-outs (Michel *et al.* 2009). In developed countries, bTB is largely managed in cattle through extensive test-and-slaughter programmes and restrictions upon herd movements (Krebs *et al.* 1997; Michel *et al.* 2009). However, these management practices can be undermined by the presence of a wildlife reservoir, with variation in badger behaviour having been repeatedly linked to changes in disease dynamics both in badgers and in cattle. For example, increased movement of badgers between social groups has been associated with an increase in disease incidence within the badger population

(Rogers *et al.* 1998; Vicente *et al.* 2007). The changes in badger spatial behaviour following culling, such as increased dispersal rates, larger range sizes, and greater social group overlap (Woodroffe *et al.* 2006; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011), has also been linked to an increase in bTB prevalence in cattle (Donnelly *et al.* 2003, 2006; Woodroffe *et al.* 2006). Variation in spatial behaviour has also been related to the infection status of the individual. For example, TB test-positive badgers are known to spend more time at outlier setts (Weber *et al.* 2013b), and have larger home ranges that overlap more with neighbouring groups compared to TB test-negative badgers (Cheeseman & Mallinson 1981; Garnett *et al.* 2005). These behaviours are likely to have implications for disease transmission. However, the extent to which these spatial behaviours are related has not been tested, so it is not known, for example, whether badgers that more frequently use outlier setts are more likely to have larger home ranges.

Through the use of diurnal and nocturnal radio tracking, this study aims to determine if badger sett use is related to badger ranging behaviour. How these spatial behaviours are associated with infection status, age, sex and season is also explored, with the implications of this behaviour for disease dynamics discussed. I predict that individuals that use outlier setts more frequently will possess larger home ranges and spend more time in neighbouring group territories, both advertised (determined by marking at latrines) and functional (determined by contact behaviour). Based on previous studies, I expect TB test-positive badgers to exhibit larger home ranges and enter neighbouring group territories more frequently. In addition, given that badgers are known to vary in their activity levels at certain times of year (Roper 2010), significant seasonal differences in ranging behaviour are also expected to be found.

4.3 Methods

Study site

Woodchester Park (N51°42' 34", W2°16' 26") is situated on the Cotswold limestone escarpment in Gloucestershire, South West England. The core study area of 7km^2 comprises of mixed woodland, pasture and arable farmland, and has a resident high-density badger population that is the subject of a long-term capture-mark-recapture study (Delahay *et al.* 2000b).

Badger sampling

Trapping events at Woodchester Park are carried out approximately 4 times a year using methods described in Delahay *et al.* 2006a. Briefly, badgers were caught using steel mesh cage traps that are baited with peanuts. They were then anaesthetised using an intramuscular administration of two parts butorphanol tartrate (Torbugesic®, Wyeth, Ontario, Canada), two parts ketamine hydrochloride (Ketaset®, Wyeth, Ontario, Canada) and one part medetomidine (Domitor®, Orion Corporation, Espoo, Finland) (De Leeuw *et al.* 2004). The capture location, sex, age and infection status were then recorded for each individual. Age is categorised as either sub-adult (>1 and <2.5 years), or adult (>=2.5 years) (Weber *et al.* 2013b). Individuals were considered TB positive if they reacted positively to either of two diagnostic tests. These were the badger-specific lateral flow antibody immunoassay (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, NY, USA), and an enzyme immunoassay for interferon-gamma (IFNγ) production in response to stimulation with purified protein derivatives of *M. bovis* and *M. avium*. When serological and cytokine assay results were combined, the sensitivity and specificity of the combined test were at least 85% and 93% respectively (Dalley *et al.* 2008; Chambers *et al.* 2009). Thirty-six badgers that were captured as part of this long-term study were collared with proximity loggers (Sirtrack, New Zealand) over 17 days between May and November 2014. The age, sex, infection status and capture locations of these individuals are summarised in Table 4.1 and Figure 4.1.

Contact events

Proximity collars recorded badger contact data between August 2014 and May 2015. These collars contained an Ultra High Frequency (UHF) transceiver that broadcasts a unique ID code whilst simultaneously ‘listening’ for those of others (Drewe *et al.* 2012). When loggers came within a defined distance, a contact was initiated and was recorded until a signal could no longer be detected (Drewe *et al.* 2012). Details of the interaction were then logged on-board the collar (Drewe *et al.* 2012). These loggers were individually set to record interactions when within 0.66+/- 0.20m of another collared individual (UHF range 5-40). This enabled interactions that occurred at close distances (e.g. fighting, grooming and mating) to be recorded, although different types of

interaction cannot be differentiated in the data. Of the 36 badgers collared, 4 had collars that were not retrieved. This was due to either the collar being dropped underground, or the badger not being recaptured at the end of the study. However, contact data were downloaded whenever badgers were recaptured throughout the year. All contacts that occurred during trapping operations and the subsequent 12 hours were omitted from the analyses. For this study, 29 badgers had sufficient contact data to be included in the analysis.

Proximity loggers have the tendency to record extended interactions as a series of shorter contacts (Drewe *et al.* 2012). Therefore, to improve the accuracy of the interaction data the protocols suggested by Drewe *et al.* 2012 were followed; all interactions that were recorded within 1.5 minutes between the same pair of loggers were amalgamated, and any additional 1-second interactions were removed. These contacts, weighted by interaction frequency, were then amalgamated into a matrix ready to be analysed using an R script (Reed 2011).

Determining advertised group ranges using bait marking

Bait marking was carried out at Woodchester Park between 19/02/2014 and 28/02/2014. This time of year was chosen due to latrine use being at its highest (Kruuk 1978), making advertised territory boundaries easier to identify. This method is described in detail in Delahay *et al.* 2000a. Briefly, baits containing small coloured plastic pellets were placed at putative main setts (Figure 4.2), with a different colour used at each. These pellets were then recovered from badger faeces during surveys of latrines. This allowed the use of each latrine to be attributed to a specific sett. Bait return data were used alongside field observations, such as well-used badger paths between adjacent territories, to infer territory boundaries. For this study, the location where each individual was trapped in relation to these mapped territorial boundaries was recorded during badger sampling, and was used to determine the “advertised” social group to which each badger belonged.

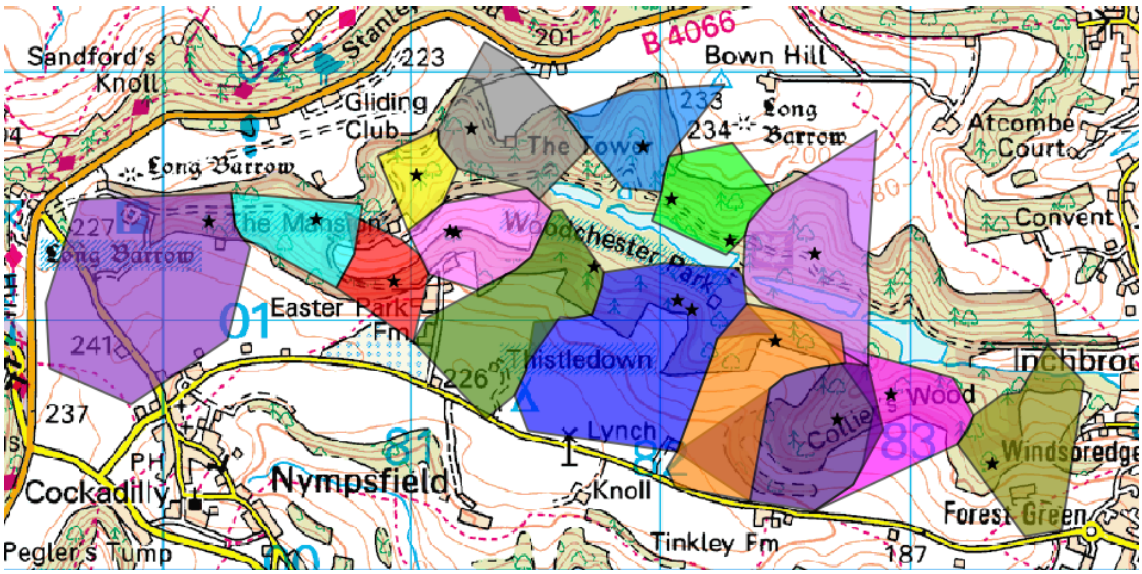


Figure 4.1 Capture locations (black stars) of 36 badgers collared in 2014 and the associated advertised territory boundaries (polygons). Polygon colour represents the following badger groups: West = purple, Larch = turquoise, Beech = red, Cedar = yellow, Boxwood = grey, Honeywell = light blue, Trackside = lime, Old Oak = lilac, Septic Tank = pink, Top/Yew = dark green, Wych Elm = dark blue, Kennel = orange, Woodrush = violet, Wood Farm = khaki.

Table 4.1 The demographic classes of the 29 badgers analysed in this study. Numbers given in brackets indicate the total number of badgers collared (36). Functional group membership was determined using modularity analysis, and the advertised group in which each badger was caught is also given.

Functional social group	Advertised spatial group	Sex		Age		Infection status	
		Male	Female	Adult	Sub-adult	Positive	Negative
Group 1	Beech	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	0 (0)
	Larch	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)
	West	0 (0)	1 (1)	0 (0)	1 (1)	1 (1)	0 (0)
Group 2	Cedar	1 (1)	2 (2)	2 (2)	1 (1)	2 (2)	1 (1)
	Septic Tank	0 (0)	1 (2)	0 (1)	1 (1)	1 (2)	0 (0)
	Breakheart	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)
Group 3	Breakheart	0 (1)	1 (1)	0 (1)	1 (1)	1 (1)	0 (1)
Group 4	Honeywell	0 (1)	1 (1)	0 (1)	1 (1)	1 (2)	0 (0)
Group 5	Trackside	1 (2)	1 (1)	0 (0)	2 (3)	2 (3)	0 (0)
Group 6	Top	1 (2)	2 (2)	1 (1)	2 (3)	1 (1)	2 (3)
	Yew	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)
Group 7	Kennel	2 (2)	2 (2)	4 (4)	0 (0)	2 (2)	2 (2)
Group 8	Colliers	2 (2)	3 (3)	4 (4)	1 (1)	4 (4)	1 (1)
	Woodrush	0 (0)	3 (3)	2 (2)	1 (1)	0 (0)	3 (3)
NA	Old Oak	0 (1)	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)
NA	Wood Farm	0 (0)	0 (1)	0 (1)	0 (0)	0 (0)	0 (1)

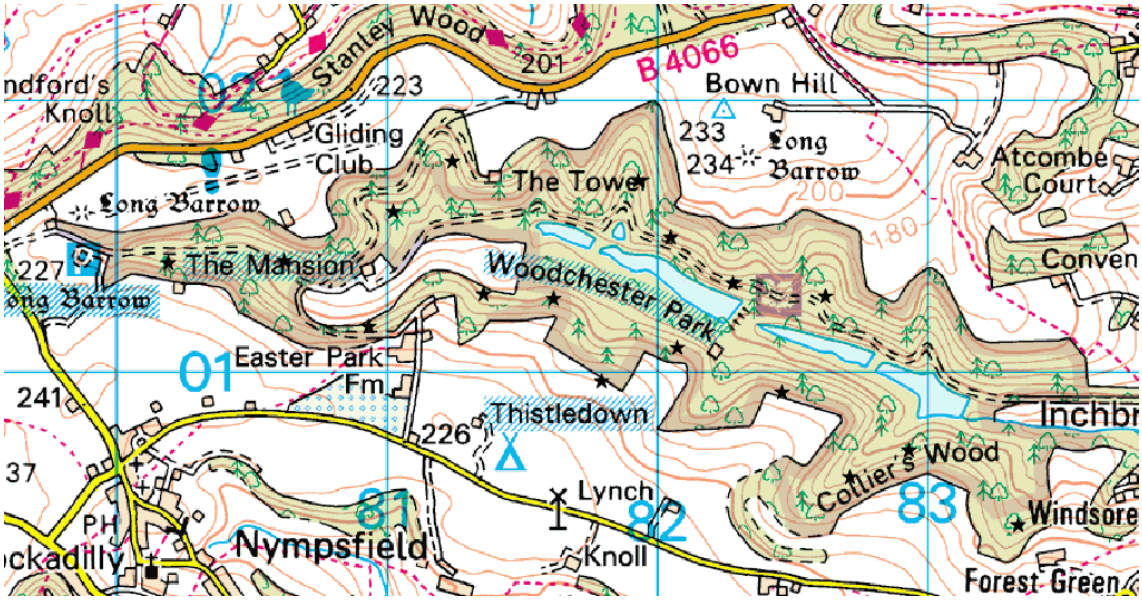


Figure 4.2 Map of Woodchester Park, the site of a long-term capture-mark-recapture study of the resident high-density badger population. Selected active main setts targeted with bait to reveal advertised territory boundaries are indicated by black stars.

Determining functional group ranges using social network analysis

Social network analysis is a quantitative tool to analyse social structure (Hawe *et al.* 2004). Networks consist of nodes (individuals) that are connected by edges (interactions), which can be binary to represent the presence or absence of interactions, or weighted to illustrate the frequency or duration of contacts. This simple representation allows many parameters to be estimated, giving a greater insight into the population than could be attained from analysing individuals in isolation (Hawe *et al.* 2004). In this study, social networks weighted by interaction frequency were built from 2014/2015 badger contact data using an established R script (Reed 2011). To identify the functional social groups present within the badger network, the network modularity (Q) was calculated using the R package 'igraph' (Csardi & Nepusz 2006). This metric is defined as the fraction of within-group edges in the observed network minus the expected fraction of within-group edges in a randomised null model (Newman 2006). This null model is based on the observed network graph, but rearranges the edges randomly with no regard to community structure (Newman 2006). Q is calculated using the following equation:

$$Q = \frac{1}{2m}\sum_{ij}\left[A_{ij} - \frac{k_i k_j}{2m}\right]\delta_{gigj}$$

(Newman 2011)

Where the expected number of edges falling between two vertices (*i* and *j*) is equal to $k_i k_j/2m$, where *k* is the node degree and *m* is the total number of edges in the observed network. The actual number of edges observed between two nodes is equal to A_{ij}. An integer label is given to each node denoting the group it belongs to in the proposed network division (g_i), and δ_{ij} is the Kronecker delta which tests whether the nodes belong to the same group (Newman 2011).

By comparing the observed to the randomised networks it can be determined whether the number of within-group edges is greater than would be expected by chance (Newman 2006), and gives a Q value that can range from -1/2 to 1. If Q<0 then no community structure is identified, but if Q=1 then highly structured communities have been detected. Q was optimised to identify natural divisions

in the network, and identify the social groups present (Newman 2006). This was done by first placing each individual in a separate community and calculating the modularity. Neighbouring communities were then joined to produce the largest increase in modularity possible. This process was repeated until the pattern of network division that gave the highest modularity score was found, and the functional social groups in the population identified (Clauset *et al.* 2004; Blondel *et al.* 2008; Verdolin *et al.* 2014). Analyses were carried out using the multi-level community function in the R package 'igraph' (Csardi & Nepusz 2006). In order to calculate territory boundaries for these functional social groups, these groups were compared to the advertised groups calculated using bait marking. Where the functional groups matched the advertised groups the same territory boundaries were maintained. However the associated territory boundaries were merged when functional groups consisted of 2 or more advertised groups. This gave approximate territories for both advertised and functional groups.

Diurnal radio tracking

In addition to UHF transceivers, badger proximity collars also contained very high frequency (VHF) radio transceivers. This enabled badgers to be located by radio tracking concurrently to proximity data being recorded. To determine if badgers were residing in main or outlier setts, badgers were located daily for 28 days per season between the hours of 08:00 and 13:00 when movement within the sett is minimal (Butler & Roper 1996). Badgers were radio tracked using a Biotrack SIKA radio-tracking receiver with a flexible Yagi antenna (Biotrack, Dorset, UK) until their position underground could be pinpointed. The type of sett they were located in (main or outlier) was then recorded. Generally, main setts had large spoil heaps and many entrances with signs of permanent habitation, whereas outlier setts were generally smaller and showed signs of less intense use (Kruuk 1978; Roper 1992). Diurnal radio tracking was repeated over three separate time periods to capture peaks in mating activity and also times where activity levels were reduced (autumn: 22/08/2014 – 19/09/2014, winter: 12/01/2015-20/02/2015, and spring: 27/03/2015- 01/05/2015) (Cresswell *et al.* 1992; Roper 2010).

Nocturnal radio tracking

Simultaneously to the diurnal tracking, badgers were radio tracked from dusk until dawn (typically 9pm-5am) using discontinuous tracking to gather independent fixes until home range asymptotes were achieved for each individual. Badger locations were mostly determined by triangulation, and supplemented with direct sightings where possible. To triangulate, compass bearings were taken in the direction of the signal source from two receiving sites with an angle difference of 45-135° (Saltz & Alkon 1985). The intersection of these bearings was considered to be the location of the individual, and were calculated using the 'Triangulation' plugin for QGIS (Jurgiel 2012). At least one hour was allowed between repeat fixes to reduce autocorrelation in the data (Huck, Davison & Roper 2008).

Variation in equipment, observer and movement of the focal individual between fixes can affect the accuracy of triangulation. Therefore the linear error between the estimate and true location was calculated prior to the start of the study. Using a method similar to Kauhala & Tiilikainen 2002, transmitters were deployed at sites commonly used in the study area, but were unknown to the observer triangulating. Half of transmitters were attached to 2 litre bottles filled with saline to mimic wave absorption and remained stationary. The remaining transmitters were attached to the ankle of a moving volunteer wearing a GPS tracker. This ensured that different levels of badger activity were represented. The linear error between the true and estimated locations was then calculated. Total linear error was 52m (+/- 46m) at distances between 10-550m. However, error dramatically increased with observer distances over 200m (0-200m = 38m +/- 36m; 200m-550m = 80m +/- 53m). Therefore, for this study the observer aimed to be within 200m of the focal individual, resulting in an average observer distance of 80m (+/- 69m).

Statistical analysis

Home range analysis

Individual home ranges for each tracking period were calculated using 95% minimum convex polygons (MCPs) using the R package 'adehabitat' (Calenge 2006). MCPs define the smallest polygon around the outermost returns where no internal angle exceeds 180 degrees (Burgman & Fox 2003). This method of

home range analysis is beneficial as it is easily comparable between studies (Harris *et al.* 1990). To quantify the degree of home range overlap with other group territories, the number of times each badger was located within and outside their group's range was determined. This was calculated for advertised and functional group ranges determined through bait marking and social network analysis respectively.

Sett use and infection status analysis

To test whether TB test-positive individuals make greater use of outlier setts than test-negative individuals, the proportion of days individuals were located at main and outlier setts were fitted against age, sex, infection status, season, and the interaction between infection status and season in a generalised linear mixed effects model with a binomial error structure. To avoid the effects of pseudoreplication, individual and group ID were included as random effects. In order to account for overdispersion in the model, an observation level random effect was also included; each data point was given a unique level of random effect to allow the extra-parametric variation in the data to be absorbed (Harrison 2015). The model was then simplified through the deletion of non-significant terms using Wald chi-square tests. All analyses were carried out using the R package "lme4" (Bates *et al.* 2014).

In the spring, TB test-negative badgers exclusively used main setts. Lack of data on outlying sett-use in this season caused convergence issues in the models: to counteract this, I conservatively included a single dummy datum representing a TB test-negative badger in an outlying sett in spring, for the sett use model only.

Home range and sett use analysis

To determine if ranging behaviour is related to sett use, range size was fitted against the proportion of time individuals were located at the main sett in a general linear mixed effects model using the R package "lme4" (Bates *et al.* 2014). Sex, infection status, age, season and their two-way interactions were included as fixed effects. Random effects were badger and group ID, to account for multiple records of each badger and the social structure of the population. To improve model fit, the response variable was square root transformed.

Competing models were then ranked using AIC values using the R package 'MuMIN' (Bartoń 2013). The parameter estimates and errors were then averaged across the entire candidate set, with each averaged parameter estimate weighted so those with low weights contribute little to the estimate (Symonds & Moussalli 2011). Significant interactions were then plotted separately whilst controlling for other variables.

The analysis was then repeated to determine if sett use correlates with home range overlap. The proportion of badger locations that occurred within and outside the advertised and functional group territory boundaries were fitted against the proportion of time individuals were located at the main sett in a general linear mixed effects model with a binomial error structure using the R package "lme4" (Bates *et al.* 2014). Sex, infection status, age, season and their two-way interactions were included as fixed effects. Random effects were badger and group ID, to account for multiple records of each badger and the social structure of the population. In order to account for overdispersion in the model, an observation level random effect was also included; each data point was given a unique level of random effect to allow the extra-parametric variation in the data to be absorbed (Harrison 2015). Competing models were then ranked using AIC values using the R package 'MuMIN' (Bartoń 2013). The parameter estimates and errors were then averaged across the entire candidate set, with each averaged parameter estimate weighted so those with low weights contributes little to the estimate (Symonds & Moussalli 2011). Significant interactions were then plotted separately whilst controlling for other variables.

4.4 Results

Infection status

Of the 36 badgers collared, 24 tested positive for bTB infection at the start of the study. A further 3 badgers tested positive during the study (n=17 female, 10 male).

Advertised and functional group ranges

Sufficient contact data were collected for 33 of the 36 collared badgers to be included in the group range analyses. The bait marking approach identified 13

advertised social groups. However 8 functional groups were identified using social network analysis (Q=0.80, Figure 4.3).

Sett use

Sufficient sett use data were collected for all 36 badgers collared. Sett use was found to dramatically vary with season, with rates of outlier use occurring orders of magnitude higher in the autumn compared to the winter and spring. However, differences in sett use were still detected between TB test-positive and TB test-negative badgers in all seasons; in the winter and spring TB test-positive badgers spent more time at outlier setts compared to TB test-negative badgers (proportion of time that TB+ badgers were found at outlier setts during the winter: 0.01 [95% CI 0.002, 0.04]; proportion of time that TB- badgers were found at outlier setts during the winter: 0.0003 [0, 0.003]; TB+ spring: 0.004 [0.0007, 0.02]; TB- spring: 0.0001 [0, 0.001]). However the opposite was true in the autumn, with TB test-negative badgers more likely to be found at outlier setts compared to TB test-positive badgers (proportion of time that TB+ badgers were found at outlier setts during the autumn: 0.31 [0.10, 0.65]; proportion of time that TB- badgers were found at outlier setts during the autumn: 0.70 [0.24, 0.95]; GLMM test of the interaction between season and infection status, X^2_3=9.95, p=0.02).

Home range size

Of the 36 badgers collared, 29 had sufficient locational data to be included in the analysis. Home range size was found to vary with season, with ranges being smaller in the winter and spring compared to the autumn (average increase in autumn range size (ha): 10.30 [95% CI 5.95, 15.84]; winter: -10.69 [-15.84, -5.95]; spring: -2.02 [-5.24, -0.38]). No difference in home range size was detected between TB test-positive and TB test-negative badgers (average increase in range size for TB test-positive badgers (ha): -0.25 [-4.08, 1.06]; TB test-negative: 0.25 [-1.06, 4.08], Figure 4.4). However, male badgers that use outlier setts more frequently were found to have smaller home ranges compared to female badgers (average decrease in home range size in males that exclusively used outlier setts (ha) = -3.50 [-10.05, -0.32]; female: 3.50 [0.34, 10.05], Figure 4.4, Figure 4.5).

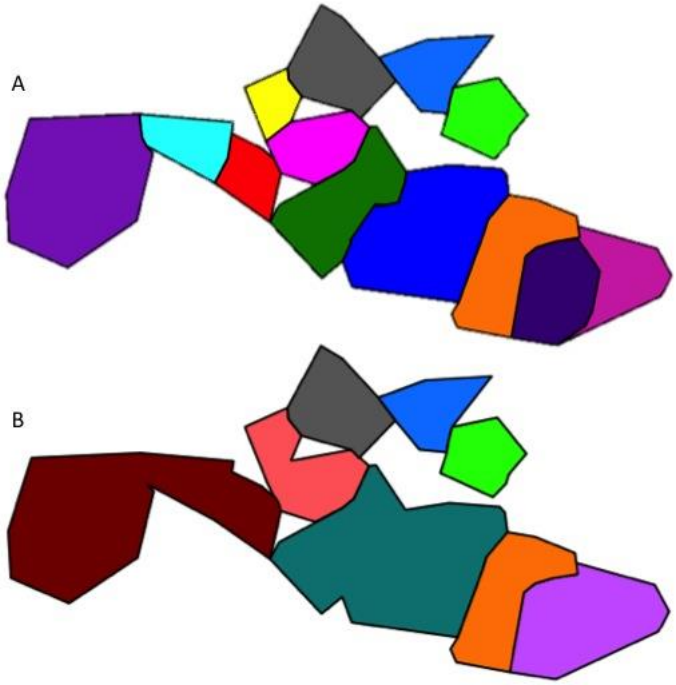


Figure 4.3 Separate group ranges determined using bait marking data to indicate the advertised group territory boundaries (A), and social network analysis to indicate the functional territory boundaries (B).

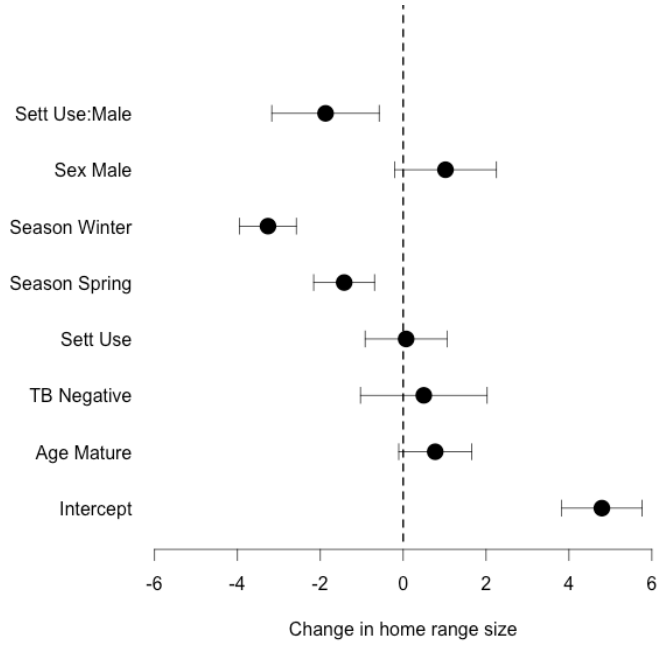


Figure 4.4 Variation in badger home range size in relation to season, sex, age, TB infection status and sett use. Values are average effects calculated across all candidate models and their 95% confidence intervals. Predictors with confidence intervals that span zero have limited explanatory power. Season and the interaction between sett use and sex have good explanatory power; home ranges are smaller in the winter, and males that use outlier setts have smaller home ranges. Infection status of the individual has low explanatory power for variation in home range size.

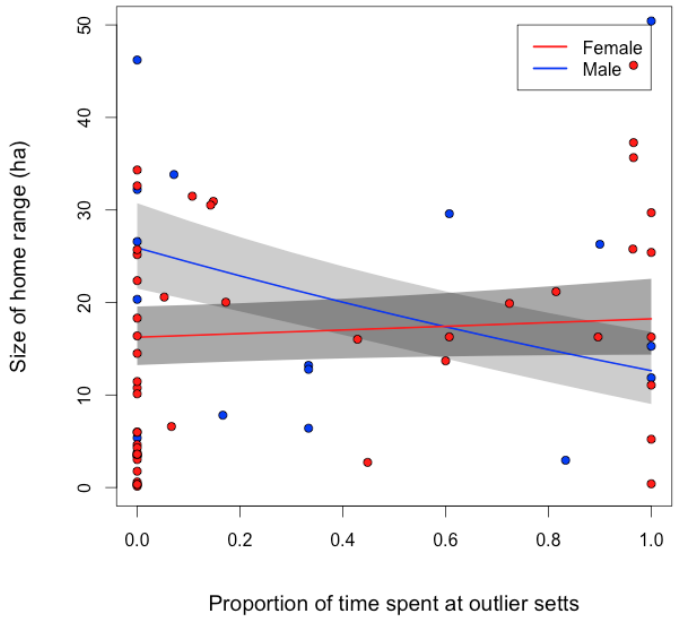


Figure 4.5 Relationship between badger home range size and proportion of time spent at outlier setts, for male and female badgers. Effects of age, infection status and season are controlled for. N=8 male and 21 female badgers. Fitted lines represent the relationship for spring. Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

Home range overlap

During the winter, badgers generally spent less time in neighbouring advertised group territories compared to the autumn and spring (Figure 4.6, Figure 4.7). However, home range overlap was also found to vary with sett use; badgers that used outlier setts more frequently in the winter and spring spent more time in neighbouring group territories (Figure 4.6, Figure 4.7). Conversely, badgers that spent more time in outlier setts in the autumn spent less time in neighbouring group territories (Figure 4.6, Figure 4.7). Home range overlap also varied with age; adult badgers that used outlier setts more frequently spent more time in neighbouring advertised group territories (Figure 4.6, Figure 4.8), and generally spent more time in other advertised territories in the autumn compared to sub-adult badgers (proportion of time adult badgers were found outside their own group territory in the autumn: 0.61 [95% CI 0.53, 0.69]; sub-adult badgers in the autumn: 0.39 [0.32, 0.47], Figure 4.6). Sex was also related to home range overlap, with males that spent more time at outlier setts spending less time in neighbouring group territories compared to females (Figure 4.6, Figure 4.9).

TB infection status was not significantly related to time spent in neighbouring advertised group territories, and consistently featured lower in the candidate model set compared to sett use (proportion of time TB- badgers were found outside their group territory: 0.37 [95% CI 0.28, 0.47]; TB+ badgers: 0.36 [0.29, 0.43], Figure 4.6, Table 4.2).

When using functional social group territories to calculate home range overlap, time spent in neighbouring group territories only varied with season; badgers spent less time in neighbouring functional group territories in the winter compared to autumn and spring (proportion of time badgers were found outside of their functional group territory in the autumn: 0.37 [95% CI 0.30, 0.44]; winter: 0.06 [0.04, 0.08]; spring: 0.29 [0.10, 0.36]). Neither sett use or infection status was related to home range overlap with functional group territories (Figure 4.10).

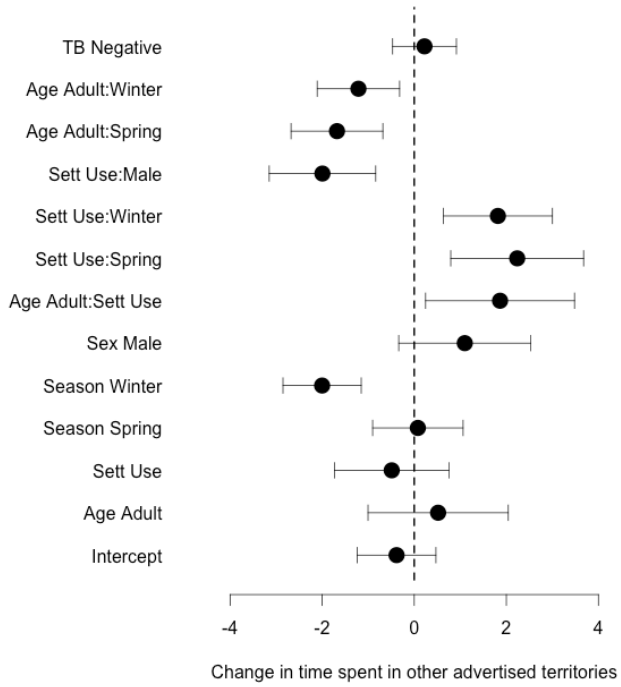


Figure 4.6 Variation in badger home range overlap with neighbouring advertised group territories in relation to season, sex, age, TB infection status and sett use. Values are average effects calculated across all candidate models and their 95% confidence intervals. Predictors with confidence intervals that span zero have limited explanatory power. Season, sett use, age and sex have good explanatory power; badgers that use outliers in winter and spring have greater home range overlap. Adult badgers that use outliers also have greater home range overlap. However males that use outliers have less home range overlap. Infection status of the individual has low explanatory power

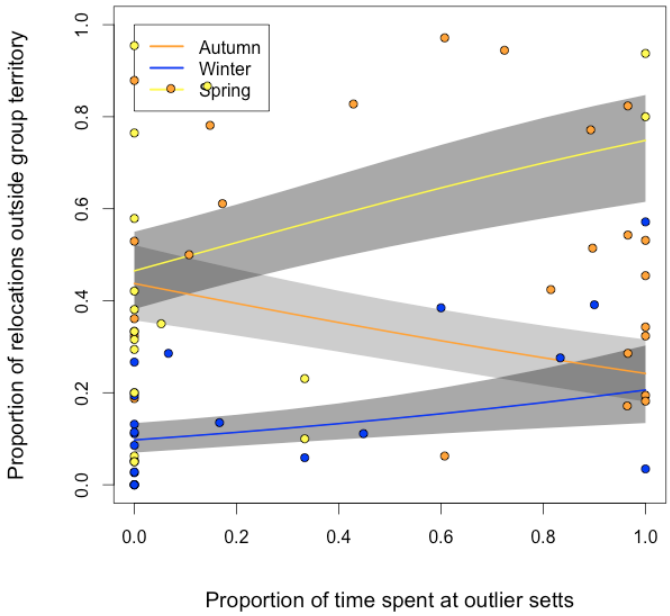


Figure 4.7 Seasonal variation in the relationship between the proportion of time spent by badgers at outlier setts and the proportion of time spent outside the badger's own social group boundaries. Boundaries were calculated using a bait marking method. Effects of age and sex are controlled for. N=26 badgers in autumn, 24 in winter and 18 in spring. Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

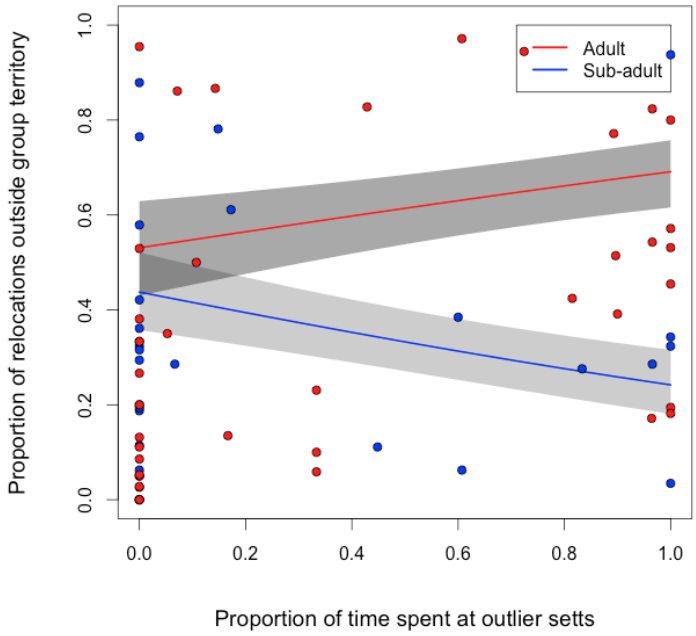


Figure 4.8 Age group variation in the relationship between the proportion of time spent by badgers at outlier setts and the proportion of time spent outside the badger's own social group boundaries. Boundaries were calculated using a bait marking method. Effects of sex and season are controlled for. N=12 sub-adult and 17 adult badgers. Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

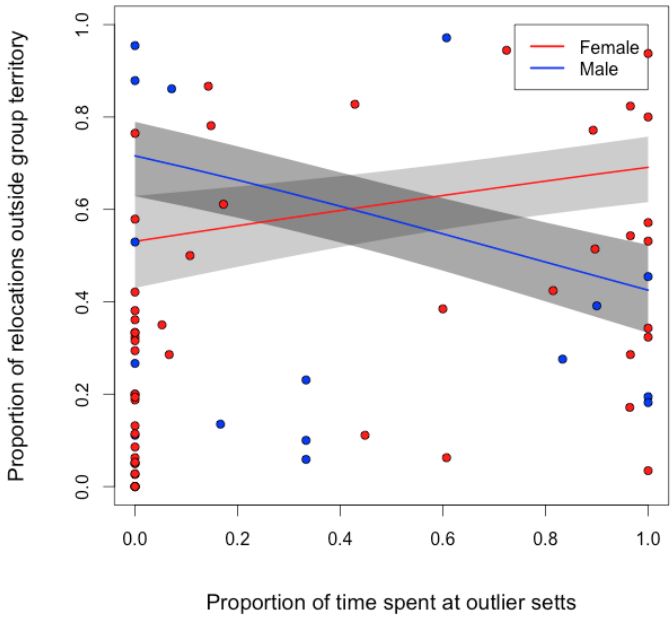


Figure 4.9 Relationship between the proportion of time spent by badgers at outlier setts and the proportion of time spent outside the badger's own social group boundaries for male and female badgers. Boundaries were calculated using a bait marking method. Effects of age and season are also controlled for. N=8 male and 21 female badgers. Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

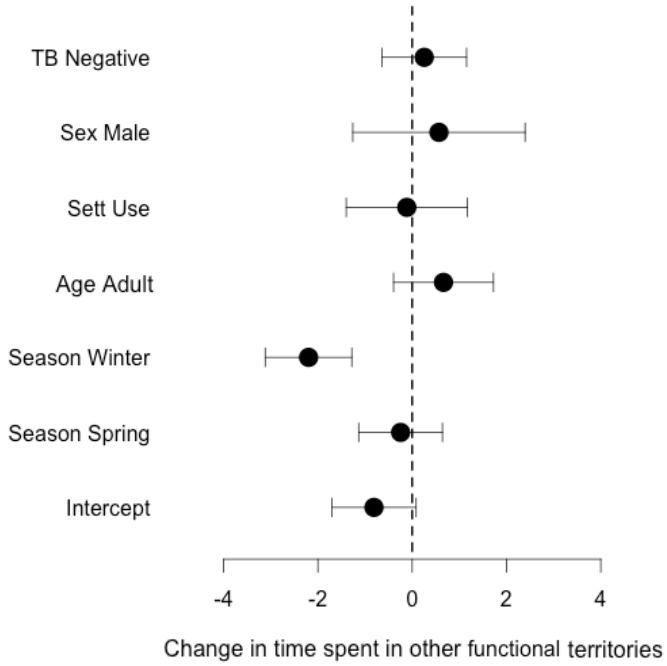


Figure 4.10 Variation in badger home range overlap with neighbouring functional social group territories in relation to season, sex, age, TB infection status and sett use. Values are average effects calculated across all candidate models and their 95% confidence intervals. Predictors with confidence intervals that span zero have limited explanatory power. Season has good explanatory power; home ranges overlap less with neighbours in the winter. Infection status and sett use have low explanatory power for change in functional home range overlap.

Table 4.2 Model selection table from the global model of predictors of home range overlap with neighbouring advertised group territories. A subset of 10 top models is shown. Sett use consistently features higher in the top model sett compared to infection status, suggesting that the infection status of the individual has less explanatory power than sett use behaviour in predicting home range overlap.

Intercept	Age	Infection	Sett	Season	Sex	Age: Infection	Age: Sett	Age: Season	Age: Sex	Infection: Sett	Infection: Season	Infection: Sex	Sett: Season	Sett: Sex	Season: Sex	**Δ**AIC	Weight
0.04	+		1.01	+	+		+						+	+		0.00	0.089
0.35	+		0.76	+	+		+	+					+	+		0.27	0.078
0.53	+		0.19	+	+			+					+	+		0.36	0.075
0.17	+		0.96	+	+		+		+				+	+		1.32	0.046
0.49	+		0.70	+	+		+	+	+				+	+		1.42	0.044
0.68	+		0.13	+	+			+	+				+	+		1.53	0.041
0.38	+		0.29	+	+		+							+		1.63	0.040
0.32	+		0.56	+				+					+			1.67	0.039
0.51	+		0.30	+	+		+		+					+		2.01	0.033
0.67	+	+	0.12	+	+			+					+	+		2.37	0.027

4.5 Discussion

This study aimed to determine if patterns of badger sett use are related to badger ranging behaviour. My results show that these behaviours are related, with badgers that frequently use outlier setts in the winter and spring spending more time in neighbouring advertised group territories. However the opposite is true in the autumn, with badgers that more frequently use outlier setts spending less time in neighbouring territories. Given that badger mating activity peaks in the spring and again to a lesser extent in the autumn (Cresswell *et al.* 1992; Roper 2010), this might suggest that these behaviours are related to badger breeding behaviour. Outlier sett use varied with TB infection status, with TB test-positive badgers using outlier setts more frequently in winter and spring when outlier sett use was generally low, and TB-test negative badgers using outliers more frequently in the autumn when outlier use was generally higher. However, infection status was not related to individual home ranging behaviour.

These findings give further insight into how badger space use varies across the year. In the autumn, badgers generally use outlier setts more frequently compared to other seasons, agreeing with a previous study on badger denning behaviour (Weber *et al.* 2013b). Badgers also have larger home ranges, consistent with suggestions that badgers need to range further due to reduced food availability (Palphramand, Newton-Cross & White 2007; Roper 2010). In addition, I also found the relationship between denning behaviour and ranging behaviour varies with season, with badgers that use outlier setts more frequently in the autumn spending more time within their own group range. However in the winter, badgers use outlier setts much less frequently, have smaller home ranges, and spend more time within their own territory boundaries. These findings are in agreement with other studies that show badgers to be less active in the winter months (Palphramand *et al.* 2007), and make greater use of the main sett perhaps for thermo-regulatory benefits (Weber *et al.* 2013b). However, counter to the autumn, badgers that use outlier setts spend more time in neighbouring group territories. Similar patterns are found in the spring, with badgers using outlier setts less frequently. However, home range sizes are larger compared to the winter, consistent with previous findings (Palphramand *et al.* 2007; Weber *et al.* 2013b). Badgers also spend

more time in neighbouring group territories in the spring, especially those that use outlier setts.

Peaks in breeding activity may explain some of these behavioural variations. Badgers that use outlier setts in the winter and spring spend more time in neighbouring territories. Badger mating activity peaks in the spring (Cresswell *et al.* 1992; Roper 2010), with extra-group mating known to commonly occur (Carpenter *et al.* 2005). Therefore, this might suggest that badgers use outlier setts as a staging point to enter neighbouring group territories from during the spring, explaining why territorial behaviour is highest at this time (Kruuk 1978; Roper & Lups 1993). A secondary, smaller peak in mating activity occurs in the autumn (Cresswell *et al.* 1992; Roper 2010), when adult badgers spend more time in neighbouring group territories. This might therefore suggest that in the autumn, only adult badgers attempt extra-group mating. Territorial behaviour may also explain why male badgers that use outlier setts have smaller home ranges that overlap less with neighbouring groups. Given that males visit boundary latrines more frequently than females (Roper *et al.* 1993), this might suggest that males spend more time defending parts of the territory boundary at this time, and so are less motivated to enter neighbouring territories.

My findings may also give some insight into the function of outlier setts. Similar seasonal differences detected in outlier sett use have been found in a previous study, with outlier setts used more in the warmer summer/autumn months compared to the colder winter/spring months (Weber *et al.* 2013b). This seasonality in sett use led to the speculation that the role of outliers may be linked to ectoparasite avoidance (Roper *et al.* 2001; Weber *et al.* 2013b), with additional theories including the provision of refuges for persecuted subordinates (Kruuk 1989), and temporary resting places to allow badgers to range further (Roper 1992). However, my findings that show how the relationship between sett use and ranging behaviour varies across seasons may indicate that the function of outlier setts changes throughout the year. In the spring, frequent use of outliers is associated with more time spent in neighbouring territories. It is intuitive to assume that this is evidence for the latter theory, with outliers used as resting places after an extraterritorial foray. However if this were the case then an increase in home range size would also

be expected with greater outlier use, given that badgers would be able to travel further. However, no evidence of this was found in this study. Instead my findings may suggest that badgers move to outliers in the spring to facilitate extra-group mating, continuing with their typical home ranging behaviour in a different location instead of using them to extend their range. This is supported by previous studies that have shown TB test-positive badgers to forage in the same habitats as TB test-negative badgers (Garnett *et al.* 2005), despite TB test-positive badgers being more likely to use outlier setts (Weber *et al.* 2013b). However, in contrast to patterns of sett usage in the spring, autumn outlier sett use was associated with less home range overlap, suggesting they may serve a different purpose at this time of year.

The relationship between sett use and ranging behaviour can offer potential insights into the spread of TB infection in badger populations. Previous studies have shown that TB test-positive badgers spend more time at outlier setts (Weber *et al.* 2013b), have larger home ranges (Garnett *et al.* 2005), and spend more time in neighbouring group territories (Cheeseman & Mallinson 1981). However, my results did not show TB infection to be significantly related to ranging behaviour. Instead, variation in patterns of sett use explained more variation in badger ranging behaviour than differences in TB infection status, with badgers that use outlier setts in the winter and spring spending more time in neighbouring territories. Given that the movement of badgers between social groups can lead to an increase in disease incidence (Rogers *et al.* 1998; Vicente *et al.* 2007), this finding might suggest that outlier sett use and the associated home range overlap may increase an individual's risk of acquiring infection. This could be from increased exposure through extra-group contacts, or increased contact with sources of environmental contamination, such as latrines. However, in order to confirm if these spatial behaviours do increase the risk of acquiring infection, a longitudinal study comparing the behaviour of badgers before and after infection would be required to directly show that badgers that exhibit these behaviours are more likely to become infected.

It has previously been shown that extra-group contact between some badger groups is so common that multiple groups effectively function as a single group (**chapter 2**). This study identified 13 advertised social groups functioning as 8

separate groups. However the relationship between sett use and home range overlap differed depending on the type of territory analysed, with badgers that more frequently used outlier setts spending more time in neighbouring advertised territories, but not functional territories. It could be possible that this is simply a product of functional territories being larger than advertised ones, giving less opportunity for home ranges to stretch over territory boundaries. However, this might also suggest that the individuals that use outlier setts and spend more time in neighbouring advertised territories have such elevated levels of extra-group contact that these advertised groups can no longer be quantitatively distinguished. This would mean that these individuals are likely to be highly influential for disease transmission among groups, and would be consistent with TB test-positive individuals being more important for disease spread between advertised groups as shown in a previous study (Weber *et al.* 2013a). However, in order for this to be determined, the relationship between home range overlap and social contacts would need to be assessed.

My findings have potential implications for wildlife disease management. Frequent outlier use is associated with greater home range overlap, and therefore higher probabilities of disease acquisition and transmission (Rogers *et al.* 1998; Vicente *et al.* 2007; Drewe 2010). Therefore, badgers that frequently use outlier setts in the spring could possibly be used as a proxy to identify high-risk individuals for disease acquisition. If individuals that use these setts at this time could be targeted with vaccination before they acquired infection, this could halt the transmission of disease to these high-risk individuals, which are likely to be important for disease spread across advertised groups and to the wider population. However, a typical territory includes multiple outlier setts (Roper 1992), and so care must be taken to ensure that all setts are included in any culling or vaccination regimes to ensure high-risk individuals are included in management activities.

Chapter 5
Badger Ranging Behaviour and Social Network Position

5.1. Abstract

1. Variation in social behaviour can have important implications for the spread of disease. Social behaviour can often be related to space use, with individuals that connect separate social groups especially important for disease transmission. European badgers (*Meles meles*) are a wildlife reservoir of bovine tuberculosis (bTB), and vary in their ranging behaviour.
2. This study aimed to establish if badger social network position is related to badger home ranging behaviour. The relationship between badger network position and home range size, and network position and home range overlap with neighbouring group territories is determined. How these relationships vary with season, infection status, sex, and age are also explored. Previous studies have shown that TB test-positive badgers have larger home ranges and are important in connecting groups. Therefore, badgers that have larger home ranges and spend more time in neighbouring group territories are expected to hold more central network positions. The implications of these findings for disease spread are also discussed.
3. Through using nocturnal radio tracking data and empirically derived contact data, I found that although network centrality is not related to home range size, it is related to home range overlap. In the spring, badgers with greater home range overlap with neighbouring group territories hold more central network positions. However badgers with greater home range overlap in the autumn are more socially isolated.
4. Given that badger breeding behaviour peaks in the spring, this might suggest that badgers are entering neighbouring group territories to achieve extra-group mating at this time. This would explain the increase in network centrality observed. However, the social isolation associated with home range overlap in the autumn may suggest the purpose of entering neighbouring group territories may change throughout the year. Behaviour that increases the connectivity of social groups is highly important for disease spread. Therefore, these findings might suggest that increased home range overlap is likely to be important for disease transmission in the spring.

5.2 Introduction

An individual's position within the social structure of a population can strongly influence the spread of infectious diseases. How connected an individual is can influence their risk of acquiring infection, and subsequently the number of individuals they can infect (Christley *et al.* 2005). Alternatively, an individual can have few contacts, but be important in connecting sub-groups, facilitating disease transmission to parts of the population that may have otherwise remained free of infection (Krause *et al.* 2007; Jones & Salathe 2010). The consequences of these individual network positions for disease spread have been empirically observed. For example, gidgee skinks (*Egernia stokesii*) that have higher contact rates have greater parasitic loads (Godfrey *et al.* 2009). Similarly, brushtail possums (*Trichosurus vulpecula*) that are better connected and bridge sub-groups in the population are more likely to acquire tuberculosis (Corner *et al.* 2003). Once infected, these social positions allow disease to quickly spread through the population (Corner *et al.* 2003).

Spatial behaviour can often be related to social behaviour (Sih *et al.* 2009). For example, African buffalo (*Syncerus caffer*) live in fission-fusion groups, but dry conditions can result in individuals switching herds more frequently, increasing group connectivity (Cross *et al.* 2004). Similarly, bolder great tits (*Parus major*) that explore their environment quickly and extensively have more contacts and connect separate groups (Aplin *et al.* 2012). Higher rates of disease transmission are thought to be a cost of group living, given that social individuals tend to experience higher contact rates compared to solitary species (Alexander 1974). However, group living has been shown to inhibit the spread of disease, as a low number of among-group contacts reduces the probability that infection will be transmitted between groups (Liu *et al.* 2003; Wu & Liu 2008; Jones & Salathe 2010). Consequently, behaviour that increases the connectivity of groups, such as those described above, are highly important for disease spread (Wu & Liu 2008; Jones & Salathe 2010). Therefore, understanding the relationship between space use and social behaviour can give important insights into disease spread through a population.

Bovine tuberculosis (bTB) is a chronic, debilitating disease of cattle, caused by the bacterium *Mycobacterium bovis*. Globally, this disease causes great

economic loss through reduced productivity, loss of trade, and compensation pay-outs (Michel *et al.* 2009). In developed countries, bTB is largely managed in cattle through extensive test-and-slaughter programmes and restrictions upon trade and animal movements (Krebs *et al.* 1997; Michel *et al.* 2009). However, in the UK, disease control was complicated by the discovery that the European badger (*Meles meles*) could carry bTB infection (Muirhead *et al.* 1974).

At medium to high densities, badgers live in mixed-sex groups (Tuyttens *et al.* 2000). These groups defend communal territories, which can remain stable for many years (Tuyttens *et al.* 2000), and are demarcated using latrines (Kruuk 1978); shallow pits in which excretory products are deposited along with scent marks from faeces, urine, and secretions from the anal, subcaudal and interdigital glands (Delahay *et al.* 2000a). Individuals within these groups do not necessarily range over the whole territory, but instead occupy their own home ranges that overlap with other group members (Kruuk 1978). Although contact rates among these social groups is generally limited (Tuyttens *et al.* 2000), individuals are known to vary in their adherence to these group territory boundaries. For example, males and older badgers are more likely to move between social groups (Rogers *et al.* 1998), and in some cases, high levels of inter-group contact can result in some groups effectively functioning as one (**chapter 2**). This can lead to the territories advertised using latrines differing from the functional boundaries.

Badger spatial behaviour has previously been related to bTB disease dynamics. For example, a change in badger group size and the movement of individuals among groups has been associated with an increase in TB incidence (Rogers *et al.* 1998; Vicente *et al.* 2007). Culling also causes an increase in the size of group territories and individual home ranges, which in turn is thought to cause an increase in contact rates between individuals, and an increase in TB prevalence (Woodroffe *et al.* 2006). TB test-positive badgers also have larger home ranges (Garnett *et al.* 2005), use outlier setts more frequently (Weber *et al.* 2013b), and are more important in bridging badger social groups compared to TB test-negative badgers (Weber *et al.* 2013a). This suggests that badgers that use outlier setts may be more important in connecting the population, and is supported by the finding that badgers that use outlier setts spend more time

in neighbouring advertised group territories (**chapter 4**). However, the relationship between ranging behaviour and network position may be complicated. For example, male badgers have consistently larger home ranges than females (Cheeseman 1979; Tuyttens *et al.* 2000), but differences in their contact rates are only evident at a specific time of year (Reed 2011). Therefore, the direct relationship between badger ranging behaviour and social behaviour warrants further exploration.

This study aims to determine if badger ranging behaviour is associated with badger social behaviour. Empirically derived social contact and radio tracking data is analysed to determine if home range size and home range overlap with neighbouring group territories is related to social behaviour. Social network analysis (SNA) is used to determine the social position of each individual in the population. Three measures of network centrality that are important for disease spread will be calculated for each individual. These are degree, closeness, and flow-betweenness. Degree is an estimate of how much direct contact an individual has in the network. This measure can indicate which individuals are at greatest risk of acquiring and transmitting infection from their direct contacts, with highly connected individuals more likely to trigger an epidemic should they become infected (Krause, Lusseau & James 2009). Closeness is a measure of how connected an individual is to the entire network, based on the mean shortest path length between the focal individual and all others. Unlike degree, this measure also includes indirect contacts. In the context of disease transmission, this means individuals with low closeness scores are more isolated and are therefore less able to acquire and transmit infection to the rest of the population. However those with high closeness scores are highly connected and more central in the network, resulting in higher risks of acquiring and transmitting infection (Corner *et al.* 2003). Flow-betweenness is a measure that reflects how important an individual is as a point of social connection based on the number of pairs of nodes an individual connects (Hawe *et al.* 2004). Individuals with high flow-betweenness will lie on many shortest paths between individuals in the network, and often bridge different groups of individuals. These influential hubs of social connection are therefore capable of spreading infection widely across the network, without necessarily needing a high number of social connections (Perkins *et al.* 2009). Together, these three centrality

measures are able to identify individuals that are influential for disease spread within the population.

Degree, closeness and flow-betweenness centrality scores are calculated for each individual using all contact events. In addition, nocturnal and diurnal contacts are analysed separately to determine if badgers hold different network positions when they are active at night compared to when they are resting during the day. Contacts that occur within and among groups are also separately analysed, using both the advertised and functional group territories.

I predict badgers with larger home ranges will have greater contact rates and hold more central network positions, given that an increase in range size is thought to increase contact rates between individuals (Woodroffe *et al.* 2006). In addition, as movement between badger groups is associated with an increase in TB incidence (Rogers *et al.* 1998; Vicente *et al.* 2007), I expect badgers with greater home range overlap with neighbouring group territories to be more important in connecting separate groups. Similar findings are expected for analyses using advertised and functional group territories. However, given that contact rates are lower between functional groups compared to advertised groups (**chapter 2**), more prominent results are expected for analysis using advertised group territory boundaries. The implications of these results for the design of effective disease control strategies are also discussed.

5.3 Methods

Study site

Woodchester Park (N51°42' 34", W2°16' 26") is situated on the Cotswold limestone escarpment in Gloucestershire, South West England. The core study area of 7km^2 comprises of mixed woodland, pasture and arable farmland, and has a resident high-density badger population that is the subject of a long-term capture-mark-recapture study (Delahay *et al.* 2000b).

Badger sampling

Trapping events at Woodchester Park are carried out approximately 4 times a year using methods described in Delahay *et al.* 2006a. Briefly, badgers were caught using steel mesh cage traps that are baited with peanuts. They were

then anaesthetised using an intramuscular administration of two parts butorphanol tartrate (Torbugesic®, Wyeth, Ontario, Canada), two parts ketamine hydrochloride (Ketaset®, Wyeth, Ontario, Canada) and one part medetomidine (Domitor®, Orion Corporation, Espoo, Finland) (De Leeuw *et al.* 2004). The capture location, sex, age and infection status were then recorded for each individual. Age is categorised as either sub-adult (>1 and <2.5 years), or adult (>=2.5 years) (Weber *et al.* 2013b). Individuals were considered TB positive if they reacted positively to either of two diagnostic tests. These were the badger-specific lateral flow antibody immunoassay (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, NY, USA), and an enzyme immunoassay for interferon-gamma (IFNγ) production in response to stimulation with purified protein derivatives of *M. bovis* and *M. avium*. When serological and cytokine assay results were combined, the sensitivity and specificity of the combined test were at least 85% and 93% respectively (Dalley *et al.* 2008; Chambers *et al.* 2009). Thirty-six badgers that were captured were collared with proximity loggers (Sirtrack, New Zealand) over 17 days between May and November 2014. The age, sex, infection status and capture locations of these individuals are summarised in Table 5.1 and Figure 4.1.

Table 5.1 The demographic classes of the 28 badgers analysed in this study. Numbers given in brackets indicate the total number of badgers collared (36). Functional group membership was determined using modularity analysis, and the advertised group in which each badger was caught is also given.

Functional social group	Advertised spatial group	Sex		Age		Infection status	
		Male	Female	Adult	Sub-adult	Positive	Negative
Group 1	Beech	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	0 (0)
	Larch	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)
	West	0 (0)	1 (1)	0 (0)	1 (1)	1 (1)	0 (0)
Group 2	Cedar	1 (1)	2 (2)	2 (2)	1 (1)	2 (2)	1 (1)
	Septic Tank	0 (0)	1 (2)	0 (1)	1 (1)	1 (2)	0 (0)
	Breakheart	0 (0)	0 (1)	0 (1)	0 (0)	0 (1)	0 (0)
Group 3	Breakheart	0 (1)	1 (1)	0 (1)	1 (1)	1 (1)	0 (1)
Group 4	Honeywell	0 (1)	1 (1)	0 (1)	1 (1)	1 (2)	0 (0)
Group 5	Trackside	1 (2)	1 (1)	0 (0)	2 (3)	2 (3)	0 (0)
Group 6	Top	1 (2)	2 (2)	1 (1)	2 (3)	1 (1)	2 (3)
	Yew	0 (0)	1 (1)	1 (1)	0 (0)	1 (1)	0 (0)
Group 7	Kennel	2 (2)	2 (2)	4 (4)	0 (0)	2 (2)	2 (2)
Group 8	Colliers	2 (2)	3 (3)	4 (4)	1 (1)	4 (4)	1 (1)
	Woodrush	0 (0)	3 (3)	2 (2)	1 (1)	0 (0)	3 (3)
NA	Old Oak	0 (1)	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)
NA	Wood Farm	0 (0)	0 (1)	0 (1)	0 (0)	0 (0)	0 (1)

Contact events

Proximity collars recorded badger contact data between August 2014 and May 2015. These collars contained an Ultra High Frequency (UHF) transceiver that broadcasts a unique ID code whilst simultaneously 'listening' for those of others (Drewe *et al.* 2012). When loggers came within a defined distance, a contact was initiated until a signal could no longer be detected (Drewe *et al.* 2012). Details of the interaction were then recorded on-board the collar (Drewe *et al.* 2012). These loggers were individually set to record interactions when within 0.66+/- 0.20m of another collared individual (UHF range 5-40). This enabled interactions that occurred at close distances (e.g. fighting, grooming and mating) to be recorded, although different types of interaction cannot be differentiated in the data. Of the 36 badgers collared, 4 had collars that were not retrieved. This was due to either the collar being dropped underground, or the badger not being recaptured at the end of the study. However, contact data were downloaded whenever badgers were recaptured throughout the year. All contacts that occurred during trapping operations and the subsequent 12 hours

were omitted from the analyses. For this study, 28 badgers had sufficient contact data to be included in the analysis.

Proximity loggers have the tendency to record extended interactions as a series of shorter contacts (Drewe *et al.* 2012). Therefore, to improve the accuracy of the interaction data the protocols suggested by Drewe *et al.* 2012 were followed; all interactions that were recorded within 1.5 minutes between the same pair of loggers were amalgamated, and any additional 1-second interactions were removed. These contacts were then amalgamated into a matrix ready to be analysed using an R script (Reed 2011).

Determining advertised group ranges using bait marking

Badger latrines consist of one or more shallow pits in a well-defined area in which excretory products are deposited (Delahay *et al.* 2000a), and are typically used to demarcate territory boundaries of badger social groups (Kruuk 1978). Bait marking was carried out at Woodchester Park between 19/02/2014 and 28/02/2014. The method is described in detail in Delahay *et al.* 2000a. Briefly, bait marking in the population studied is carried out each spring when latrine use is at its highest (Kruuk 1978). This along with low vegetation levels makes territory boundaries easier to identify at this time. Each territory typically has an associated main sett which is permanently occupied and used by all members of a social group (Roper 1992). Baits containing peanuts, syrup and small coloured indigestible plastic pellets were placed at these setts (Figure 4.2), with a differently coloured or shaped pellet used at each. These pellets were then recovered from badger faeces during surveys for latrines, allowing the use of each latrine to be attributed to a specific sett. These data were used alongside field observations, such as well-used badger paths between adjacent territories, to infer territory boundaries for each social group. For this study, the location where each individual was trapped in relation to these mapped territorial boundaries was recorded during badger sampling, and was used to determine the “advertised” social group to which each badger belonged.

Determining functional group ranges using social network analysis

Social network analysis is a quantitative tool to analyse social structure (Hawe *et al.* 2004). Networks consist of nodes (individuals) that are connected by

edges (interactions), which can be binary to represent the presence or absence of interactions, or weighted to illustrate the frequency or duration of contacts. This simple representation allows many parameters to be estimated, giving a greater insight into the population than could be attained from analysing individuals in isolation (Hawe *et al.* 2004). In this study social networks were built from contact data using an R script which amalgamates contacts into a matrix ready to be analysed (Reed 2011).

To identify the functional social groups present within the badger network, the network modularity (Q) was calculated using the R package 'igraph' (Csardi & Nepusz 2006). This metric is defined as the fraction of within-group edges in the observed network minus the expected fraction of within-group edges in a randomised null model (Newman 2006). This null model is based on the observed network graph, but rearranges the edges randomly with no regard to community structure (Newman 2006). Q is calculated using the following equation:

$$Q = \frac{1}{2m}\sum_{ij}\left[A_{ij} - \frac{k_i k_j}{2m}\right]\delta_{gigj}$$

(Newman 2011)

Where the expected number of edges falling between two vertices (*i* and *j*) is equal to $k_i k_j/2m$, where *k* is the node degree and *m* is the total number of edges in the observed network. The actual number of edges observed between two nodes is equal to A_{ij}. An integer label is given to each node denoting the group it belongs to in the proposed network division (g_i), and δ_{ij} is the Kronecker delta which tests whether the nodes belong to the same group (Newman 2011).

By comparing the observed to the randomised networks it can be determined whether the number of within-group edges is greater than would be expected by chance (Newman 2006), and gives a Q value that can range from -1/2 to 1. If $Q<0$ then no community structure is identified, but if $Q=1$ then highly structured communities have been detected. Q was optimised to identify natural divisions in the network, and identify the functional social groups present (Newman

2006). This was done by first placing each individual in a separate community and calculating the modularity. Neighbouring communities were then joined to produce the largest increase in modularity possible. This process was repeated until the pattern of network division that gave the highest modularity score was found, and the functional social groups in the population identified (Clauset *et al.* 2004; Blondel *et al.* 2008; Verdolin *et al.* 2014). All analyses took into account the edge weight, which was taken as the frequency of interactions, and were carried out using the multi-level community function in the R package 'igraph' (Csardi & Nepusz 2006). In order to calculate territory boundaries for these functional social groups, these groups were compared to the advertised groups calculated using bait marking. Where the functional groups matched the advertised groups the same territory boundaries were maintained. However the associated territory boundaries were merged when functional groups consisted of 2 or more advertised groups. This gave approximate territories for both advertised social groups and also functional social groups.

Radio tracking

In addition to UHF transceivers, badger proximity collars also contained very high frequency (VHF) radio transceivers. This enabled badgers to be located by radio tracking concurrently to proximity data being recorded. Badgers were discontinuously radio tracked from dusk until dawn (typically 9pm-5am) until home range asymptotes were achieved for each individual. Badger locations were mostly determined by triangulation, and supplemented with direct sightings where possible, using a Biotrack SIKA radio-tracking receiver with a flexible Yagi antenna (Biotrack, Dorset, UK). To triangulate, compass bearings were taken in the direction of the signal source from two receiving sites with an angle difference of 45-135° (Saltz & Alkon 1985). The intersection of these bearings was considered to be the location of the individual, and were calculated using the 'Triangulation' plugin for QGIS (Jurgiel 2012). At least one hour was allowed between repeat fixes to reduce autocorrelation in the data (Huck *et al.* 2008). This was repeated over 3 separate time periods to capture peaks in mating activity and also times where activity levels were reduced (autumn: 22/08/2014 - 19/09/2014, winter: 12/01/2015 - 20/02/2015, and spring: 27/03/2015 - 01/05/2015) (Cresswell *et al.* 1992; Roper 2010).

Variation in equipment, observer and movement of the focal individual between fixes can affect the accuracy of triangulation. Therefore the linear error between the estimate and true location was calculated prior to the start of the study. Using a method similar to Kauhala & Tiilikainen 2002, transmitters were deployed at sites commonly used in the study area, but were unknown to the observer triangulating. Half of transmitters were attached to 2 litre bottles filled with saline to mimic wave absorption and remained stationary. The remaining transmitters were attached to the ankle of a moving volunteer wearing a GPS tracker. This ensured that different levels of badger activity were represented. The linear error between the true and estimated locations was then calculated. Total linear error was 52m (+/- 46m) at distances between 10-550m. However, error dramatically increased with observer distances over 200m (0-200m = 38m +/- 36m; 200m-550m = 80m +/- 53m). Therefore for this study the observer aimed to be within 200m of the focal individual, resulting in an average observer distance of 80m (+/- 69m).

Statistical analysis

Home range analysis

Individual home ranges for each tracking period were calculated using 95% minimum convex polygons (MCPs) using the R package 'adehabitat' (Calenge 2006). This method defines the smallest polygon around the outermost returns where no internal angle exceeds 180 degrees (Burgman & Fox 2003), and is beneficial as it is easily comparable between studies (Harris *et al.* 1990). To quantify the degree of home range overlap with other group territories, the number of times each badger was located within and outside their group's range was determined. This was calculated for advertised and functional group ranges. Advertised ranges were determined through bait marking, and functional group ranges were determined using social network analysis and merging the corresponding bait marking group boundaries.

Social network analysis

Social networks were built from the amalgamated contact data. Given that the frequency and duration of contact events recorded by proximity loggers are known to be highly correlated (Reed 2011), but contact duration deemed slightly more accurate (Drewe *et al.* 2012), contacts weighted by duration were

used in this analysis. A different network was created for each season to correspond with the radio tracking data. These were autumn (22/08/2014 – 21/11/2014), winter (22/11/2014 – 21/02/2015) and spring (22/02/2015 – 21/05/2015). These seasonal networks are referred to as the complete networks. Complete networks were then divided temporally into diurnal (6am-8pm) and nocturnal (8pm-6am) networks. This allowed contacts that occurred when badgers were resting during the day to be distinguished from contacts that occurred at night when badgers were foraging etc. Networks were also created to distinguish between within- and among-group contacts, for group membership determined using both the advertised and functional territory boundaries. However, during the day when badgers were resting, the majority of contacts occurred between members of the same social group. Therefore, only the complete and within-group networks were analysed separately for diurnal contacts.

Three social network centrality metrics were calculated for each individual per network. These were degree, closeness and flow-betweenness. Degree is a measure of how much direct contact an individual has with others in the network. Given that repeated exposure to infection is likely to be necessary for successful TB transmission (Porphyre *et al.* 2011), for this analysis degree was weighted by the total duration of contacts between individuals. Closeness is a measure of distance, using an individual's direct and indirect contacts to reflect how many steps it takes to reach all others in the network. Flow-betweenness is a measure of how important an individual is as a point of social connection, based on the number of times an individual connects nodes that otherwise would not have been able to reach each other. Each of these centrality measures is associated with an increased chance of acquiring infection (Christley *et al.* 2005). For this analysis the R package "tnet" (Opsahl 2015) was used to calculate degree and closeness scores, and flow-betweenness was calculated using the R package "sna" (Butts 2014).

Social network models

To determine if network position was related to range size, the network metrics were fitted separately against range size in a general linear mixed effects model using the R package "lme4" (Bates *et al.* 2014). Age, disease status, season,

and their two-way interactions with range size were included as fixed effects. Random effects were badger and advertised spatial group ID, to account for multiple records of each badger and the spatial structure of the population. To improve model fit, the response variable was square root transformed, or if zero inflated log(y+1) transformed. Given that network data is non-independent, a permutation-based approach to statistical inference is regarded to be the most appropriate approach to analysis (Croft *et al.* 2011). Therefore, the values of the response variable were permuted among individuals within each season. Permutations were carried out 1000 times, and models were fitted to each of these 1000 permuted datasets. Model estimates from the observed data were then compared to the distribution of estimates from the permuted datasets, and considered to be significant if the estimate from the observed data fell *outside* of the 95% confidence intervals of the null estimates.

The analysis was then repeated to determine if network position was related to home range overlap. The three centrality measures were fitted separately against the proportion of badger locations that occurred within and outside the advertised group territory in a general linear mixed effects model, using the R package “lme4” (Bates *et al.* 2014). Sex, disease status, age, season and their two-way interactions with home range overlap were included as fixed effects. Random effects were badger and group ID. To improve model fit, the response variable was square root transformed, or log(y+1) transformed if it was zero inflated. The values of the response variable were permuted among individuals within each season 1000 times, and models were fitted to each of these 1000 permuted datasets. Model estimates from the observed data were then compared to the distribution of estimates from the permuted datasets, and considered to be significant if the estimate from the observed data fell *outside* of the 95% confidence intervals of the null estimates. This home range overlap analysis was also repeated using the proportion of badger locations that occurred within and outside the functional group territories.

Some advertised badger groups had only one individual collared, and so within-group contacts were unable to be recorded. Therefore these individuals were removed from the overall and within-group centrality models only. All individuals were included in the among-group analysis.

5.4 Results

Community detection

Sufficient contact data for analysis were collected for 33 of the 36 badgers collared. The bait marking approach identified these 33 individuals to reside in 13 advertised social groups. However 8 functional groups were identified using social network analysis (Q=0.80, Figure 5.1).

Home range size and network position

Sufficient locational and contact data were collected for 28 of the 36 badgers collared. Network position was not related to home range size generally, or in any particular season. Badgers with larger home ranges did not differ in their degree scores (average increase in sqrt(degree) per unit increase in home range size (ha): -3.4 [95% CI from permuted data -10.1, 8.6]), their closeness scores (average increase in sqrt(closeness) per unit increase in home range size (ha): -0.01 [-0.05, 0.04]), or their betweenness scores (average increase in sqrt(betweenness) per unit increase in home range size (ha): 0.04 [-0.19, 0.15]. Due to similarities in results across all networks, only the estimates from the complete network are shown. Confidence intervals are calculated from empirical null distributions, with estimates considered significant if they fell outside of these intervals).

Home range overlap and network position

Advertised territories

Badgers with home ranges that overlapped with neighbouring advertised group territories held different network positions compared to those that remained within their own group boundaries. In the spring, badgers that spent more time in neighbouring advertised territories were generally more central in the network, having larger degree, closeness and flow-betweenness scores (Tables 5.2 - 5.4, Figure 5.2). This was true for the complete and the nocturnal network. In the diurnal network, degree and closeness scores were found to be higher for those that spent more time in neighbouring territories, but no effect on betweenness scores were observed (Tables 5.2 - 5.4). However the opposite was true in the autumn; badgers that spent more time in neighbouring group territories were generally more isolated, having lower degree, closeness and flow-betweenness scores in the complete and nocturnal network (Tables 5.2 -

5.4, Figure 5.2). This was also found in the diurnal network but to a lesser extent, where badgers that spent more time in neighbouring territories had lower degree and closeness scores (Tables 5.2 and 5.3). Badgers that spent more time in neighbouring group territories in the winter were not found to differ in their network positions (Tables 5.2 - 5.4).

Network position also varied when within- and among-group contacts were analysed separately. Badgers that spent more time in neighbouring territories had lower within-group betweenness scores in the diurnal network across the whole year (Table 5.4). Similar results were found in the autumn, where badgers that spent more time in neighbouring territories had lower within-group degree and closeness scores in the complete, nocturnal and diurnal networks (Tables 5.2 and 5.3). However in the spring, badgers that spent more time in neighbouring territories had higher within-group degree and closeness scores (Tables 5.2 and 5.3). No differences in betweenness scores were found (Table 5.4). No differences in network position were detected for badgers that had greater home range overlap in the winter (Tables 5.2 - 5.4), or in the among-group networks in any season (Tables 5.2 - 5.4).

Functional territories

Similar results were found when analysing home range overlap with functional territories. In the spring, badgers that spent more time in neighbouring territories had higher overall degree and closeness scores for the complete and nocturnal networks (Tables 5.5 and 5.6, Figure 5.3). In the diurnal network only closeness scores were higher (Table 5.6). However in the autumn the opposite was true, with badgers that spent more time in neighbouring territories having lower overall degree and closeness scores for the complete and nocturnal networks (Tables 5.5 and 5.6, Figure 5.3), and lower closeness scores in the diurnal network (Table 5.6). No differences in network position were found for badgers that had greater home range overlap in the winter (Tables 5.5 – 5.7, Figure 5.3).

Within-group network position was also found to vary. Badgers that spent more time in neighbouring territories in the spring had higher within-group degree scores for the complete and diurnal network (Table 5.5), and within-group closeness scores were higher for the complete, nocturnal and diurnal network

(Table 5.6). Conversely, badgers that spent more time in neighbouring territories in the autumn had lower within-group degree scores for the complete and diurnal network (Table 5.5), and within-group closeness scores were lower for the complete, nocturnal and diurnal network (Table 5.6). No effect on flow-betweenness was detected (Table 5.7), and home range overlap with functional groups was not related to among-group network position in any season (Tables 5.5 – 5.7).

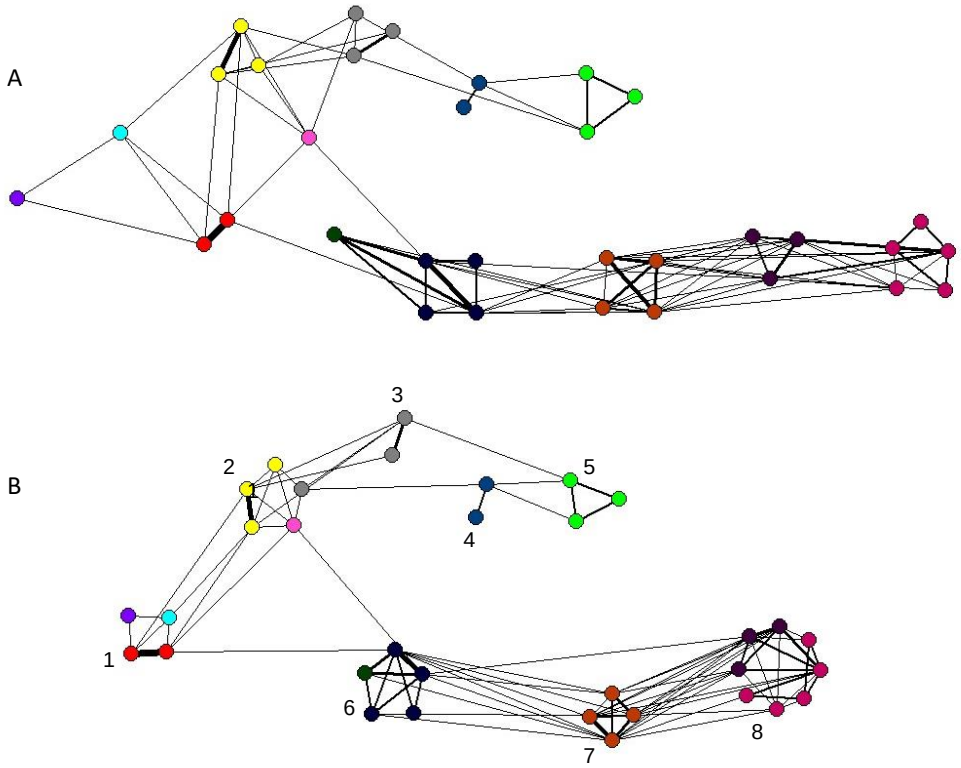


Figure 5.1 Network diagrams of sampled badgers (n=33) divided according to the method of community detection. Communities are arranged corresponding to the spatial location of the main sett, but the proximity of nodes to each other is of no relevance. Line thickness is proportional to interaction frequency and nodes are coloured according to the coloured marker in the bait. A = the thirteen advertised spatial groups identified using the bait marking approach, B = the eight functional social groups identified using the network modularity approach. Numbers represent the functional group number.

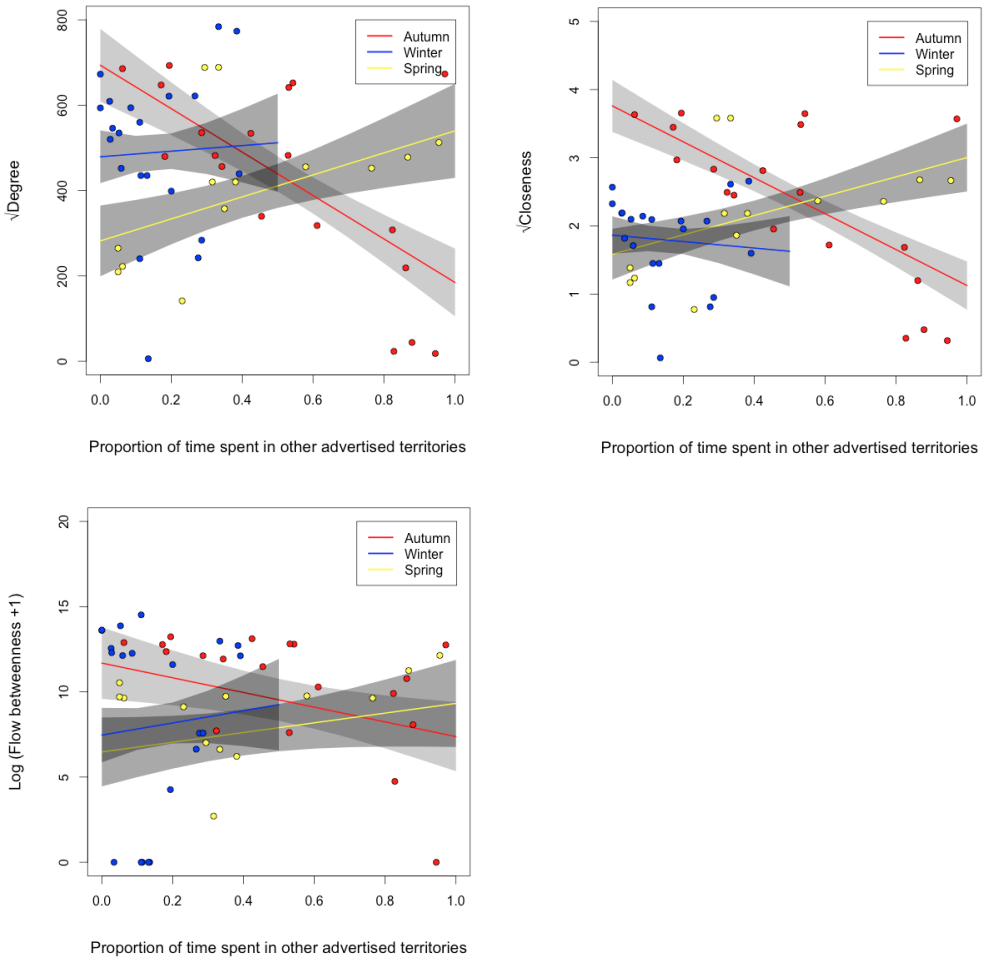


Figure 5.2 Relationship between badger home range overlap with neighbouring advertised territories calculated using the bait marking method, and social network position. Social network position calculated using all contact events are shown. Results for degree centrality weighted by contact duration, closeness centrality, and flow-betweenness centrality are shown separately for the autumn (n=19), winter (n=21) and spring (n=13). Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

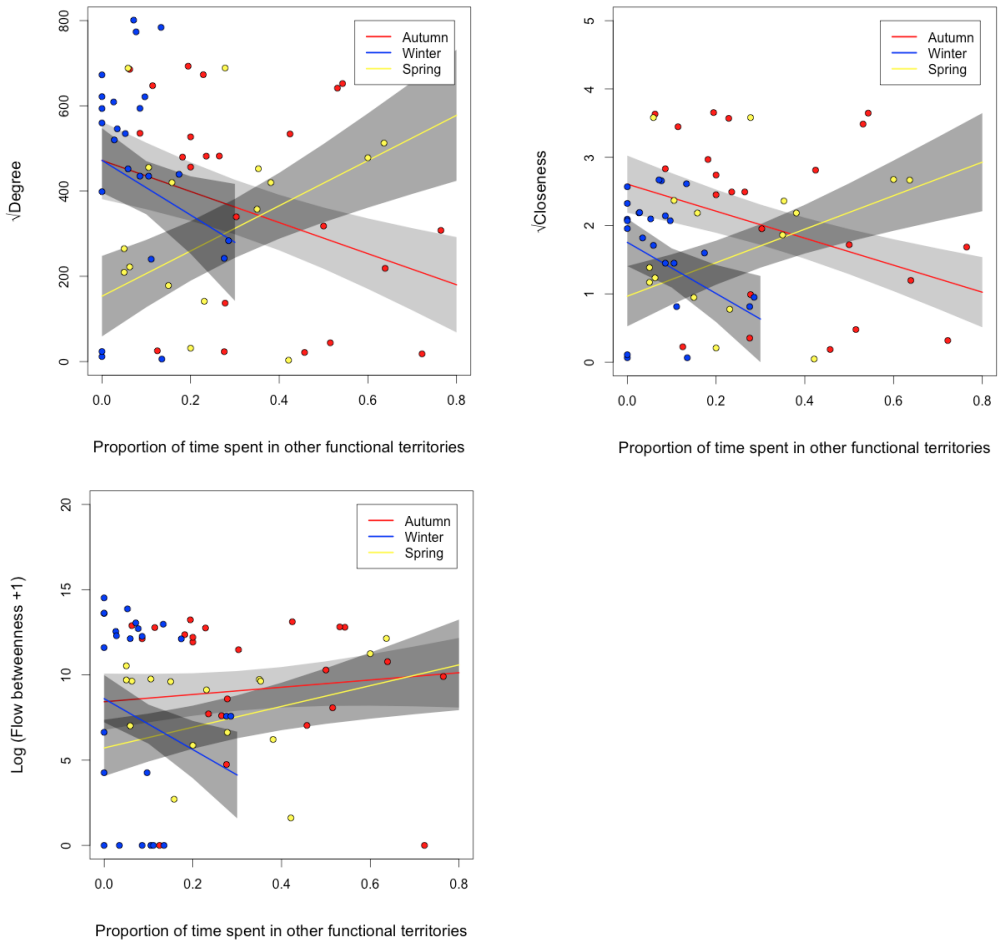


Figure 5.3 Relationship between badger home range overlap with neighbouring functional territories calculated using the modularity approach, and social network position. Social network position calculated using all contact events are shown. Results for degree centrality weighted by contact duration, closeness centrality, and flow-betweenness centrality are shown separately for the autumn (n=23), winter (n=24) and spring (n=16). Shaded areas signify the standard error around the fitted values. Data points represent the raw data.

Age and network position

Social network position varied depending on the age of the individual. Adult badgers had higher betweenness scores compared to sub-adult badgers in the complete, the nocturnal and the diurnal networks (Table 5.4). No relationship between age and within-group network position was detected, but among advertised groups adult badgers had higher betweenness scores compared to sub-adult badgers in the nocturnal network (Table 5.4). Age was not related to network position in any of the networks analysed using functional social groups (Tables 5.5 – 5.7).

Sex and network position

Social network position was also related to sex. Males had lower closeness scores overall and within advertised groups in the nocturnal network (Table 5.3). Males also had lower nocturnal within-group degree scores compared to females (Table 5.2). However, sex was not related to network position in any of the networks analysed using functional social groups (Tables 5.5 – 5.7).

TB and network position

TB test-positive badgers had lower among-advertised-group flow-betweenness scores compared to TB test-negative badgers in the nocturnal network (Table 5.4). However TB status was not related to network position in any of the networks analysed using functional social groups (Tables 5.5 – 5.7).

Season and network position

Overall network position was also found to vary with season. Compared to the autumn, winter centrality scores were generally lower. In the complete network, degree and closeness scores were lower overall, and within advertised groups (Tables 5.2 and 5.3). Similarly, in the nocturnal network, closeness scores were lower overall and within advertised groups (Table 5.3). Within-group betweenness scores were also lower (Table 5.4), as were among-group degree scores (Table 5.2). In the diurnal network, overall closeness scores were lower in the winter (Table 5.3), as were overall and within-group degree scores (Table 5.2).

In the spring, centrality scores were also lower compared to the autumn. In the diurnal network, overall betweenness scores were lower (Table 5.4), as were degree scores within functional groups in the nocturnal network (Table 5.5).

Table 5.2 Model outputs of degree centrality fitted against individual home range overlap with advertised territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the dataset, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group degree were square root transformed, and among-group degree was log(y+1) transformed. All and within-group networks: N=24, among-group network: N=28.

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	**744 (163, 699)**	**631 (136,572)**	10 (5,12)	**307 (69, 292)**	**257 (44,237)**	**12 (7,11)**	**16 (6, 14)**	11 (4,14)
Range overlap (autumn)	**-718 (-712,687)**	**-557 (-510,505)**	-1 (-8,8)	**-263 (-236, 228)**	-207 (-221,215)	**-6 (-5,6)**	**-12 (-10, 9)**	**-13 (-12, 11)**
Spring	**-439 (-262,219)**	**-386 (-182,279)**	-5 (-6,0)	**-110 (-64, 142)**	**-85 (-15,164)**	-2 (-2,3)	**-5 (-3, 5)**	**-4 (-2, 7)**
Winter	**-245 (-162,287)**	**-156 (-113,305)**	-3 (-6,1)	18 (-9, 217)	54 (30, 219)	**-1 (-1,3)**	**-4 (-3, 5)**	**-2 (-2, 7)**
Adult	30 (-202,203)	44 (-180,193)	1 (-4,4)	19 (-116, 117)	28 (-103, 103)	0 (-2, 2)	0 (-3, 3)	0 (-3, 3)
Male	-202 (-235,235)	-178 (-206,224)	2 (-5,5)	-134 (-141, 129)	**-130 (-129, 110)**	-2 (-3,2)	-4 (-4, 3)	-2 (-4, 3)
Infected	-8 (-215, 214)	16 (-196,198)	-3 (-4,4)	-45 (-122, 131)	-36 (-112, 107)	0 (-2,2)	1 (-3, 3)	1 (-4, 3)
Overlap:Spring	**815 (-502,499)**	**886 (-492,491)**	2 (-7,7)	**276 (-216, 207)**	**310 (-191, 200)**	4 (-6,5)	**12 (-7, 7)**	**13 (-8, 8)**
Overlap:Winter	751 (-803, 765)	465 (-700,704)	7 (-15,15)	260 (-454, 462)	123 (-416,396)	5 (-8,7)	12 (-13, 12)	7 (-15, 12)
Overlap:Adult	76 (-557,572)	35 (-526,564)	-2 (-8,8)	1 (-303, 322)	-17 (-270, 258)	4 (-5,5)	2 (-9, 9)	7 (-10, 10)
Overlap:Male	299 (-447, 443)	155 (-451,431)	-1 (-8,8)	188 (-222, 242)	133 (-186, 212)	4 (-5,5)	7 (-7,7)	-3 (-8 8)
Overlap:Infected	61 (-596,593)	-42 (-528,522)	3 (-9,9)	78 (-326, 324)	43 (-267, 278)	-3 (-6,5)	-2 (-8, 9)	-5 (-9, 12)

Table 5.3 Model outputs of closeness centrality fitted against individual home range overlap with advertised territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the dataset, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group closeness were square root transformed, and among-group closeness was log(y+1) transformed. All and within-group networks: N=24, among-group network: N=28.

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	**4.0 (1.1, 3.6)**	**2.2 (0.4,2.0)**	1.2 (0.0,2.4)	**4.1 (1.5,3.7)**	**2.1 (0.5, 2.0)**	2.2 (0.7, 2.5)	**2.1 (0.5, 1.9)**	1.1 (-0.3, 1.5)
Range overlap (autumn)	**-3.7 (-3.2,3.2)**	**-1.9 (-1.9,1.8)**	-0.4 (-2.8,2.9)	**-3.2 (-2.4,2.4)**	-1.6 (-1.7, 1.6)	-1.8 (-2.1, 2.1)	**-2.2 (-1.7,1.7)**	-1.3 (-1.5, 1.4)
Spring	**-2.4 (-1.4,0.9)**	**-1.4 (-0.7,0.9)**	-1.0 (-1.8,0.9)	**-2.0 (-1.4, 0.8)**	**-1.1 (-0.6, 0.8)**	-1.1 (-1.2, 0.6)	**-1.5 (-0.9, 0.7)**	**-0.9 (-0.6, 0.7)**
Winter	**-2.2 (-1.7,0.6)**	**-0.9 (-0.7,0.8)**	-0.8 (-1.5,0.6)	**-2.2 (-1.9, 0.3)**	**-0.7 (-0.6, 0.7**)	-1.0 (-1.1, 0.4)	**-0.8 (-0.7, 0.7)**	-0.4 (-0.5, 0.7)
Adult	0.2 (-0.8,0.9)	0.2 (-0.6,0.6)	-0.1 (-0.9,0.8)	0.2 (-0.8, 0.8)	0.2 (-0.6, 0.5)	-0.1 (-0.7, 0.7)	0.2 (-0.6, 0.7)	-0.1 (-0.5, 0.5)
Male	-0.9 (-0.9,1.0)	-0.6 (-0.6,0.7)	-0.2 (-1.2,1.2)	**-1.0 (-0.9, 1.0)**	**-0.7 (-0.6, 0.7)**	-0.8 (-0.9, 0.9)	-0.5 (-0.7, 0.7)	-0.3 (-0.5, 0.5)
Infected	0.1 (-0.9,0.9)	-0.1 (-0.6,0.6)	0.1 (-0.9,0.9)	-0.1 (-0.9, 0.8)	-0.1 (-0.6, 0.6)	-0.1 (-0.7, 0.7)	-0.0 (-0.6, 0.6)	-0.2 (-0.5, 0.5)
Overlap:Spring	**4.4 (-2.3,2.5)**	**3.0 (-1.6,1.7)**	1.3 (-2.5,2.8)	**3.4 (-2.4, 2.2)**	**2.5 (-1.4, 1.5)**	1.7 (-1.8, 1.9)	**2.8 (-1.6, 1.6)**	**2.0 (-1.5, 1.3)**
Overlap:Winter	3.3 (-3.5,3.4)	2.0 (-2.4,2.4)	0.9 (-3.3,3.2)	3.2 (-3.3, 3.5)	1.6 (-2.2, 2.2)	1.9 (-2.3, 2.3)	1.7 (-2.6, 2.2)	1.0 (-2.0, 1.9)
Overlap: Adult	0.3 (-2.6,2.5)	0.2 (-1.8,1.8)	0.4 (-2.1,2.4)	0.2 (-2.4, 2.3)	0.1 (-1.6, 1.7)	1.0 (-1.7, 1.8)	0.1 (-1.8, 1.8)	0.6 (-1.6, 1.6)
Overlap:Male	1.5 (-2.2,2.1)	0.5 (-1.4,1.4)	0.6 (-2.1,2.3)	1.7 (-2.1, 1.8)	0.6 (-1.3, 1.3)	1.3 (-1.7 1.9)	0.9 (-1.4, 1.4)	0.2 (-1.1, 1.1)
Overlap:Infected	0.1 (-2.9,2.6)	-0.2 (-1.8,1.8)	-0.5 (-2.4,2.4)	0.2 (-2.5, 2.4)	-0.1 (-1.7, 1.7)	-0.6 (-1.9,1.8)	0.1 (-1.7, 1.8)	-0.5 (-1.5, 1.4)

Table 5.4 Model outputs of flow-betweenness centrality fitted against individual home range overlap with advertised territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the data, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group flow-betweenness were square root transformed, and among-group flow-betweenness was log(y+1) transformed. All and within-group networks: N=24, among-group network: N=28.

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	12 (6, 15)	13 (2,14)	8 (3,11)	11 (5,13)	12 (1, 12)	10 (5, 12)	11 (3, 15)	11 (1, 13)
Range overlap (autumn)	-3 (-9,9)	**-17 (-15,13)**	2 (-9,8)	-2 (-8,8)	-15 (-17, 17)	-2 (-7, 8)	-7 (-13,12)	**-14 (-13, 13)**
Spring	**-7 (-5,3)**	-7 (-9,4)	-4 (-6,1)	**-5 (-5, 2)**	-6 (-6, 3)	-4 (-4, 2)	**-7 (-7, 4)**	-7 (-8, 4)
Winter	-6 (-6,3)	-6 (-7,4)	-6 (-8,-1)	-5 (-6, 3)	**-5 (-5, 3)**	-4 (-5,2)	-5 (-8, 4)	-5 (-7, 5)
Adult	**6 (-5,5)**	3 (-6,6)	3 (-4,4)	**5 (-5, 5)**	2 (-5, 5)	**4 (-4, 4)**	**7 (-6, 6)**	3 (-6, 6)
Male	0 (-6,6)	-2 (-7,7)	0 (-4,5)	0 (-5, 6)	-2 (-6, 6)	0 (-5, 5)	-1 (-7, 7)	0 (-7, 7)
Infected	-5 (-5,6)	-4 (-7,6)	-1 (-4,3)	-4 (-5, 5)	-3 (-5, 4)	**-3 (-3,4)**	-5 (-6, 6)	-5 (-6, 6)
Overlap:Spring	**9 (-8,8)**	11 (-13,13)	2 (-7,7)	**7 (-7, 6)**	9 (-16, 16)	5 (-7, 7)	11 (-12, 12)	11 (-13, 13)
Overlap:Winter	13 (-21,22)	13 (-23,24)	3 (-12,14)	13 (-20, 19)	12 (-30, 30)	11 (-15, 15)	9 (-25, 24)	9 (-24, 23)
Overlap: Adult	-11 (-13,12)	2 (-15,15)	-4 (-8,8)	-10 (-12, 11)	2 (-19, 20)	-5 (-8,8)	-6 (-16, 15)	1 (-16, 17)
Overlap:Male	1 (-8,10)	5 (-13,12)	-2 (-9,8)	1 (-9, 9)	5 (-15, 16)	0 (-8, 8)	1 (-12, 13)	1 (-12, 11)
Overlap:Infected	8 (-12,12)	5 (-16,17)	-2 (-8,8)	6 (-12, 12)	4 (-16, 16)	2 (-9,9)	6 (-17, 15)	5 (-16, 17)

Table 5.5 Model outputs of degree centrality fitted against individual home range overlap with functional territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the dataset, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group degree were square root transformed, and among-group degree was log(y+1) transformed. N=28.

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	574 (134,633)	574 (135,632)	7 (2,8)	270 (46,275)	270 (41,275)	6 (2,8)	13 (6,14)	13 (6,14)
Range overlap (autumn)	-720 (-832, 856)	-726 (-866, 823)	-1 (-11, 10)	-331 (-370, 357)	-344 (-357, 365)	-1 (-10, 9)	-10 (-16, 14)	-10 (-16, 15)
Spring	**-331** **(-282,194)**	**-330** **(-272, 170)**	-3 (-5,0)	**-91** **(-75, 128)**	**-88** **(-80, 127)**	-3 (-5, 0)	-4 (-4, 4)	-4 (-4, 4)
Winter	-32 (-118, 291)	-34 (-116, 271)	-4 (-5, 0)	68 (18, 198)	70 (25, 206)	-4 (-5, 0)	-2 (-3, 4)	-2 (-3, 4)
Adult	-84 (-178, 208)	-86 (-213, 199)	1 (-3, 3)	-31 (-107, 115)	-31 (-103, 116)	1 (-3, 3)	-1 (-3, 4)	-1 (-3, 4)
Male	-8 (-237, 211)	-7 (-267, 241)	1 (-4,4)	-38 (-140, 134)	-39 (-145, 117)	2 (-3, 3)	0 (-5, 4)	0 (-4, 4)
Infected	-13 (-191, 204)	-12 (-204, 210)	-2 (-3, 3)	-44 (-102, 105)	-47 (-112, 111)	2 (-3, 2)	0 (-3, 4)	0 (-3, 4)
Overlap:Spring	**949** **(-803, 791)**	**949** **(-744, 750)**	1 (-8, 8)	**348** **(-388, 330)**	348 (-355, 349)	1 (-8, 8)	13 (-13, 13)	**13** **(-14, 12)**
Overlap:Winter	19 (-1450, 1287)	26 (-1311, 1365)	7 (-20, 20)	36 (-818, 764)	29 (-851, 732)	8 (-18, 21)	2 (-26, 23)	2 (-29, 21)
Overlap: Adult	563 (-864, 764)	565 (-830, 812)	-2 (-8, 8)	238 (-400, 410)	224 (-425, 371)	-2 (-7, 8)	6 (-14, 14)	6 (-14, 14)
Overlap:Male	-126 (-707, 719)	-125 (-762, 758)	-9 (-10, 10)	-4 (-353, 347)	4 (-350, 368)	-9 (-10, 10)	-1 (-13, 13)	-1 (-13, 13)
Overlap:Infected	-167 (-747, 738)	-167 (-749, 753)	3 (-10, 9)	-27 (-326, 355)	-6 (-365, 343)	1 (-9, 8)	-3 (-14, 14)	-3 (-12, 14)

Table 5.6 Model outputs of closeness centrality fitted against individual home range overlap with functional territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the dataset, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group closeness were square root transformed, and among-group closeness was log(y+1) transformed. N=28.

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	3.0 (1.0,3.2)	2.2 (-0.7,2.4)	1.2 (0.2,2.0)	3.2 (1.3,3.4)	2.3 (0.8,2.4)	1.2 (0.2,2.0)	1 .5 (0.3,1.8)	1.4 (0.4,1.6)
Range overlap (autumn)	-3.5 (-4.2, 4.0)	-2.7 (-3.1, 2.9)	0.1 (-3.0, 3.2)	-3.2 (-4.0, 3.9)	-2.5 (-2.9, 2.6)	0.2 (-3.0, 3.3)	-2.0 (-2.4, 2.5)	-1.9 (-2.4, 2.4)
Spring	**-1.7 (-1.5, 0.9)**	**-1.1 (-0.9, 0.8)**	-0.6 (-1.3, 0.3)	**-1.5 (-1.5, 0.6)**	**-0.9 (-0.8, 0.8)**	-0.6 (-1.3, 0.3)	**-1.1 (-0.8, 0.6)**	**-1.0 (-0.8, 0.5)**
Winter	-1.0 (-1.4, 0.5)	-0.4 (-0.8, 0.6)	-0.9 (-1.5, 0.1)	-1.3 (-1.6, 0.3)	-0.5 (-0.8, 0.6)	-1.0 (-1.5, 0.2)	-0.2 (-0.5, 0.7)	-0.2 (-0.5, 0.6)
Adult	-0.4 (-0.9, 0.8)	-0.3 (-0.6, 0.6)	0.3 (-0.7, 0.7)	-0.4 (-0.8, 0.8)	-0.3 (-0.7, 0.7)	0.2 (-0.7, 0.6)	-0.2 (-0.6, 0.6)	-0.2 (-0.5, 0.6)
Male	-0.1 (-1.1, 1.0)	-0.1 (-0.8, 0.8)	0.0 (-0.8, 0.9)	-0.1 (-1.0, 1.0)	-0.2 (-0.8, 0.8)	0.1 (-0.9, 0.9)	-0.0 (-0.8, 0.7)	-0.0 (-0.7, 0.7)
Infected	0.1 (-0.8, 0.8)	0.1 (-0.7, 0.7)	-0.3 (-0.6, 0.7)	-0.0 (-0.8, 0.8)	-0.1 (-0.6, 0.6)	-0.3 (-0.7, 0.7)	0.1 (-0.5, 0.6)	0.1 (-0.5, 0.5)
Overlap:Spring	**4.7 (-4.1, 3.9)**	**3.6 (-2.9, 3.0)**	0.1 (-2.7, 2.6)	**3.7 (-3.5, 3.5)**	**2.8 (-3.0, 2.7)**	0.1 (-2.7, 2.5)	**3.2 (-2.4, 2.5)**	**3.0 (-2.3, 2.2)**
Overlap:Winter	-0.2 (-5.9, 5.3)	-0.4 (-4.4, 4.6)	0.9 (-4.0, 4.6)	1.1 (-5.7, 5.2)	0.3 (-4.7, 4.4)	1.7 (-4.3, 4.5)	-1.1 (-3.8, 3.9)	-0.8 (-3.6, 3.4)
Overlap: Adult	2.9 (-3.6, 3.8)	2.3 (-2.9, 2.7)	0.4 (-2.4, 2.0)	3.1 (-3.5, 4.0)	2.1 (-2.7, 2.8)	0.6 (-2.4, 2.1)	1.5 (-2.4, 2.4)	1.6 (-2.2, 2.4)
Overlap:Male	-0.3 (-3.3, 3.2)	-0.2 (-2.4, 2.7)	-1.3 (-2.7, 2.8)	-0.4 (-3.2, 3.4)	-0.1 (-2.3, 2.4)	-1.5 (-2.5, 2.7)	-0.3 (-2.1, 2.2)	-0.3 (-2.1, 2.1)
Overlap:Infected	-1.4 (-3.4,3.3)	-1.0 (-2.8, 2.9)	-0.8 (-2.7, 2.5)	-1.7 (-3.5, 3.4)	-0.9 (-2.4, 2.5)	-1.0 (-2.8, 2.7)	-0.6 (-2.2, 2.0)	-0.7 (-2.1, 2.1)

Table 5.7 Model outputs of flow-betweenness centrality fitted against individual home range overlap with functional territories. Age, disease status, season, and their two-way interactions with home range overlap are also included in the model. Observed model estimates are shown, with 95% confidence intervals given in brackets. Confidence intervals were calculated from the distribution of 1000 model estimates fitted to 1000 permutations of the dataset, with observed model estimates considered to be significant if they fell *outside* of the 95% confidence intervals. Model estimates for the interaction between home range overlap and season represent differences from the autumn estimates. Significant values are shown in bold. Models for the complete, nocturnal and diurnal networks are shown for the overall, within- and among-group contact events. To improve model fit, the estimates for overall and within-group flow-betweenness were square root transformed, and among-group flow-betweenness was log(y+1) transformed. N=28

	Complete Network			Nocturnal Network			Diurnal Network	
	Overall	Within Groups	Among Groups	Overall	Within Groups	Among Groups	Overall	Within Groups
Intercept	9 (5,14)	8 (5,14)	6 (2,8)	8 (5,13)	7 (4,12)	6 (2,8)	7 (2,13)	7 (2,14)
Range overlap (autumn)	4 (-10, 9)	5 (-16, 16)	-1 (-11, 10)	5 (-13, 12)	6 (-14, 13)	0 (-11, 10)	-2 (-20, 19)	-1 (-20, 19)
Spring	-3 (-6,2)	-2 (-6, 2)	-4 (-5, 1)	-2 (-4, 2)	-2 (-5, 2)	-4 (-5, 1)	-3 (-7, 3)	-3 (-7, 3)
Winter	0 (-6, 3)	1 (-6, 2)	-5 (-7, -1)	0 (-5, 2)	1 (-5, 2)	-5 (-7, -2)	1 (-6, 4)	0 (-6, 4)
Adult	3 (-5, 5)	4 (-5,5)	1 (-2, 2)	3 (-4, 4)	3 (-4, 4)	1 (-2, 2)	4 (-5, 5)	4 (-5, 5)
Male	2 (-6, 5)	1 (-6, 6)	0 (-3, 3)	2 (-5, 5)	1 (-5, 5)	0 (-3, 3)	0 (-7, 7)	1 (-6, 6)
Infected	-3 (-5,5)	-4 (-5, 5)	-1 (-2, 2)	-3 (-4, 4)	-3 (-4, 4)	-1 (-2, 2)	-3 (-5, 5)	-4 (-5, 5)
Overlap:Spring	4 (-8, 8)	2 (-13, 14)	5 (-9, 9)	2 (-10, 10)	1 (-12, 11)	5 (-9, 10)	6 (-16, 17)	5 (-17, 18)
Overlap:Winter	-14 (-18, 17)	-26 (-35, 32)	0 (-13, 15)	-10 (-30, 27)	-23 (-30, 28)	1 (-12, 16)	-21 (-36, 34)	-18 (-39, 38)
Overlap: Adult	-6 (-13, 12)	-5 (-16, 18)	-1 (-7, 7)	-6 (-14, 12)	-6 (-13, 14)	-1 (-8, 8)	1 (-19, 19)	1 (-18, 19)
Overlap:Male	-3 (-9, 9)	-2 (-16, 15)	-5 (-10, 9)	-4 (-13, 12)	-1 (-13, 14)	-5 (-9, 8)	0 (-19, 18)	-1 (-17, 17)
Overlap:Infected	3 (-13,12)	2 (-14, 16)	-2 (-8, 9)	2 (-12, 12)	1 (-12, 13)	-3 (-8, 9)	2 (-18, 19)	2 (-18, 19)

5.5 Discussion

This study aimed to determine whether badger social network position is related to ranging behaviour. In the autumn, badgers that spend more time in neighbouring group territories are more socially isolated in the network. However in the spring, badgers with greater home range overlap hold more central network positions. Given that these central network positions are associated with an increase in disease acquisition and transmission in other populations (Corner *et al.* 2003; Christley *et al.* 2005; Godfrey *et al.* 2009), this suggests that badgers that venture outside their own territory boundaries are likely to be more important for disease spread in the spring, but contribute little in the autumn. Home range size is not related to network position, with badgers that have larger home ranges holding similar network positions to those that do not range as far. This suggests individuals that increase the connectivity of badger groups are likely to be more influential for disease dynamics, compared to those that range over wider areas.

These findings can give further insight into how badger behaviour changes with season. In the autumn, badgers that spend more time in neighbouring group territories are generally more isolated in the network, having fewer contacts and generally holding less central network positions. However in the winter, network position was not related to home range overlap, but centrality scores were generally lower at this time. This may be linked to badgers being less active in the winter months, possibly due to reduced food availability (Palphramand *et al.* 2007; Roper 2010). Conversely, badgers that spend more time in neighbouring group territories in the spring hold more central network positions. This timing coincides with a known peak in mating activity (Cresswell *et al.* 1992; Roper 2010).

Badger breeding behaviour could help explain the seasonal differences detected in these results. In the spring, individuals that spend more time in neighbouring territories have higher contact rates and are more connected to the network at night when they are active. This timing coincides with a peak in badger mating activity (Cresswell *et al.* 1992; Roper 2010), and territoriality (Kruuk 1978; Roper & Lups 1993), with observations of immigrant males being chased away from the main sett at this time (Christian 1995). Given that extra-

group mating is common in badger populations, possibly to avoid inbreeding (Da Silva *et al.* 1994; Carpenter *et al.* 2005; Dugdale *et al.* 2008; Annavi *et al.* 2014, **chapter 3**), this might suggest that badgers are entering neighbouring group territories in the spring to increase mating opportunities. If this were the case, then contact with both prospective mates, and through aggressive encounters, might explain the observed increase in network centrality. This theory is further supported by findings that adult badgers are more likely to hold these network positions, given that they are more likely to partake in mating activity.

However in the autumn, different network positions are associated with home range overlap, suggesting that the purpose of extra-territorial ranging may change throughout the year, in a similar way to outlier sett use (**chapter 4**). In the autumn, badgers with greater home range overlap have fewer contacts and are more socially isolated. This coincides with a secondary, smaller peak in mating activity (Cresswell *et al.* 1992; Roper 2010), and a peak in bite wounding in males (Cresswell *et al.* 1992). Given that only 27% of males are estimated to breed in a given year (Dugdale *et al.* 2007), it could be speculated that these network positions associated with home range overlap may suggest that mating predominantly occurs within groups in the autumn, with intra sex conflict between males leading to the exclusion of subordinates to the edges of the territory. Although persecution has led to subordinates exhibiting different ranging strategies in other species (e.g. Wauters & Dhondt 1992; Boydston *et al.* 2003; Murray, Mane & Pusey 2007), this theory is highly speculative, and more research into badger mating behaviour and the seasonality of space use is needed.

In the spring, badgers with greater home range overlap also hold more central network positions during the day when they are resting. These individuals have higher contact rates, are better connected to their own group members, and are important in connecting different groups within the diurnal network. These network positions when den sharing have previously been associated with an increase in bTB acquisition in captive possums, leading to faster disease transmission through the population (Corner *et al.* 2003). In addition, given that the poor respiratory conditions experienced within badger setts are likely to

increase the risk of aerosol transmission (Roper 1992), these resting network positions are likely to be highly important for disease transmission. However, the opposite is true in the autumn, with individuals that spend more time in neighbouring territories more socially isolated in the diurnal network. This is likely to further reduce the contribution that these individuals make to disease transmission at this time.

In general, individuals that spend more time in neighbouring advertised and functional group territories hold similar network positions. However some differences can be observed. For advertised territories, badgers with greater home range overlap in the spring are important in connecting different communities within the population. However this difference is not detected when functional boundaries are analysed. This suggests that these individuals are likely to be highly influential for disease transmission among advertised groups, but less so among functional groups. These differences may indicate that these individuals with greater home range overlap connect advertised groups to the extent that they function as a single group, therefore drastically reducing the protection group living can offer from disease invasion (Jones & Salathe 2010). However, given that functional group overlap is associated with generally higher levels of centrality that are also known to be important for disease transmission (Corner *et al.* 2003; Christley *et al.* 2005; Godfrey *et al.* 2009; Bull *et al.* 2012), individuals that hold these network positions are still likely to be important for disease spread across functional groups.

Home range overlap is not related to network position among badger groups. This is surprising given that home range overlap has been associated with an increase in rabies transmission in jackals (*Canis mesomelas* and *Canis adustus*) (Loveridge & Macdonald 2001), and TB transmission in meerkats (*Suricata suricatta*) (Drewe 2010). Given that sex, age and TB status have previously been related to among-group network positions in badgers (Reed 2011; Weber *et al.* 2013a), home range overlap would be expected to be the mechanism facilitating this contact. This lack of result could possibly be caused by a lack of power in my analysis, with my sample size being smaller than that used in the Weber *et al*. 2013a study. This could make relatively rare among-group contact events even harder to detect. Similar issues regarding power

were experienced in a previous study that used sample sizes similar to that of my own (Goodman 2007).

In general, my results show that TB test-positive badgers do not differ in their network position compared to TB test-negative badgers, counter to a previous study (Weber *et al.* 2013a). However, I did find badger ranging behaviour to be strongly related to network positions that have previously been shown to increase the risk of acquiring disease (Corner *et al.* 2003; Godfrey *et al.* 2009; Bull *et al.* 2012). Therefore, my results may suggest that home range overlap is better able to explain network position compared to TB status. This would be consistent with a previous finding that outlier sett use is a better predictor of home range overlap than TB status (**chapter 4**), and may suggest that these spatial behaviours increase an individuals likelihood of acquiring infection, rather than infection driving variations in network position as has been previously theorised (Cheeseman & Mallinson 1981; Garnett *et al.* 2005). However, as discussed, the smaller sample size used in my study may have reduced the ability to detect behavioural differences associated with TB status. Therefore a longitudinal study to determine if badger behaviour becomes more abnormal with disease progression, or if individuals that exhibit certain behaviours are more likely to acquire infection, is required.

My results show that home range overlap, but not home range size, is related to social network position. This suggests that home range overlap is likely to be more important for disease transmission than home range size. This finding was surprising given that badgers that range more widely in response to culling have increased contact rates, and therefore disease transmission (Woodroffe *et al.* 2006). Previous studies have also shown that TB test-positive badgers range further than TB test-negative badgers, which was attributed to an increased exposure to infectious agents (Garnett *et al.* 2005). However, Garnett *et al*. 2005 also found TB test-positive badgers to have greater home range overlap than TB test-negative badgers, but this result was not discussed. This might suggest that home range overlap increases exposure to infectious agents, not home range size as has been previously assumed. Further, increased home range overlap has repeatedly been reported in studies of social perturbation (Tuyttens *et al.* 2000; Woodroffe *et al.* 2006; Carter *et al.* 2007). Therefore, my

results could indicate that home range overlap might be more influential for disease transmission than home range size, through increasing connectivity in the network. This could provide insight into the mechanism that allows social perturbation to increase disease transmission amongst badger populations.

Social network analysis can identify individuals that are important for disease transmission, allowing individuals to be targeted for a more efficient disease control strategy (Grassly & Fraser 2008; Jones & Salathe 2010). This study found that badgers with greater home range overlap are possibly more important for disease transmission in the spring compared to the autumn. Therefore, if control could be targeted towards these individuals in the spring before they acquired infection, this has the potential to significantly reduce transmission rates for the whole population. These individuals are also more likely to use outlier setts at this time (**chapter 4**). Therefore, outlier sett use in the spring could potentially offer a spatial proxy to identify these high-risk individuals. This strategy is likely to be more effective than random vaccination (Litvak-Hinenzon & Stone 2009). Similarly, these individuals could be selectively removed from the population. However, given that even small-scale badger removals have been shown to cause social disruption (Bielby *et al.* 2014), vaccination might be a more reliable strategy. However, territories typically contain multiple outlier setts (Roper 1992), that can be hard to identify (Robertson *et al.* 2017). Therefore the logistic feasibility of this management strategy requires further investigation. Social network analysis can play an important role in increasing the efficacy of disease management strategies, however network data is not always available. Therefore, our findings suggest that spatial proxies could be used to target individuals that are likely to be important for disease spread.

Chapter 6
General Discussion

6.1 Overview

This thesis aimed to explore different elements of community structure within a European badger (*Meles meles*) population, and their implications for bovine tuberculosis (bTB) disease transmission. In this final chapter, I will discuss how this research has contributed to three general areas: badger social structure and behaviour, the implications of social structure for disease transmission, and disease management. Suggestions for further areas of research are also discussed.

6.2 Badger social structure and behaviour

The results of this thesis can give insight into the social structure of the badger population. Within a population, communities can form when variation in contact rates lead some individuals to interact more with each other than with the rest of the population (Newman 2002; Oliveira & Gama 2012). The strength of this community structure depends on the amount of among-group contact that occurs; individuals that predominantly interact with their own group increase community isolation and the strength of the community structure, whereas those that seek extra-group contacts make these communities less distinct (Clauset *et al.* 2004). At medium to high densities, badgers live in social groups with limited among-group contact rates (Tuyttens *et al.* 2000), and so a strong community structure is expected to be present. In **chapter 2**, I found this to be the case, with badgers interacting within very distinct sub-groups. However, extra-group contact was detected between all groups identified, and in some cases extra-group contact was so common that some groups could no longer be quantitatively distinguished (**chapter 2**).

To further understand the population social structure, and gain insight into behaviours that are important for connecting communities, I investigated how badger sett use, ranging, and social behaviour changes throughout the year. Although aspects of these behaviours have previously been investigated, I show how the relationships between these behaviours change across seasons. These relationships are summarised in Figure 6.1. In the autumn, badgers generally range over larger areas, and use setts away from the social group's main sett more frequently (**chapter 4,** Figure 6.1). This is consistent with previous findings, with badgers thought to range further due to limited food

availability (Palphramand *et al.* 2007; Roper 2010), and to use outlier setts for reasons linked to ectoparasite avoidance (Roper *et al.* 2001; Weber *et al.* 2013b). Badgers that use these outlying setts have similar home range sizes to those that use the main sett (**chapter 4**). However, badgers that use the main sett spend more time in neighbouring territories (**chapter 4**) and are subsequently more socially isolated (**chapter 5,** Figure 6.1). In addition to limited food availability, small peaks in mating activity and male bite wounding also occur in the autumn (Cresswell *et al.* 1992; Roper 2010). Therefore, an increase in resource competition may lead to the exclusion of some individuals to the edges of the territory, explaining the observed home range overlap and associated social isolation (**chapter 4, chapter 5**, Figure 6.1).

In the winter, badgers have much smaller home ranges and spend more time at the main sett (**chapter 4,** Figure 6.1). This is also consistent with previous studies that show badgers to be less active in the winter months (Palphramand *et al.* 2007), and make greater use of the main sett possibly for thermo-regulatory benefits (Weber *et al.* 2013b). However, counter to the autumn, badgers that use outlying setts during the winter spend more time in neighbouring territories, but do not differ in their network positions (**chapter 4, chapter 5**, Figure 6.1).

In the spring, badgers use the main sett more frequently and have larger home ranges (**chapter 4**, Figure 6.1). Badgers generally spend more time in neighbouring territories (**chapter 4**, Figure 6.1), particularly those that make use of outlier setts. These badgers spend the majority of their time in neighbouring group territories, and hold more central network positions, having higher contact rates and being better connected in the network (**chapter 4, chapter 5**, Figure 6.1). Badger mating behaviour and territoriality is known to peak in the spring (Kruuk 1978; Cresswell *et al.* 1992; Roper & Lups 1993; Roper 2010), with extra-group mating known to commonly occur (Carpenter *et al.* 2005). Therefore, it is possible that badgers might use outlier setts in the spring as a staging point to enter neighbouring group territories. This could explain why home range overlap is associated with higher contact rates, given that badgers may encounter greater levels of aggression in addition to the contacts that occur directly as a product of mating.

These seasonal differences in badger behaviour suggest that the function of outlier setts and extra-territorial ranging may change throughout the year. The purpose of outlier setts is generally unknown, with theories including ectoparasite avoidance (Roper *et al.* 2001), a refuge for persecuted subordinates (Kruuk 1989), or as a resting place when foraging away from the main sett (Roper 1992). My results might suggest that in the spring badgers use outlier setts and spend more time in neighbouring territories to facilitate extra-group mating, possibly to avoid inbreeding. This theory is supported by evidence that extra-group contact is more likely to occur between less related individuals (**chapter 3**), and that adult badgers are more important in bridging social groups (**chapter 2**). However, in the autumn extra-group ranging is associated with social isolation. This could suggest that some individuals are being socially excluded, given that resource competition is likely to be high at this time.

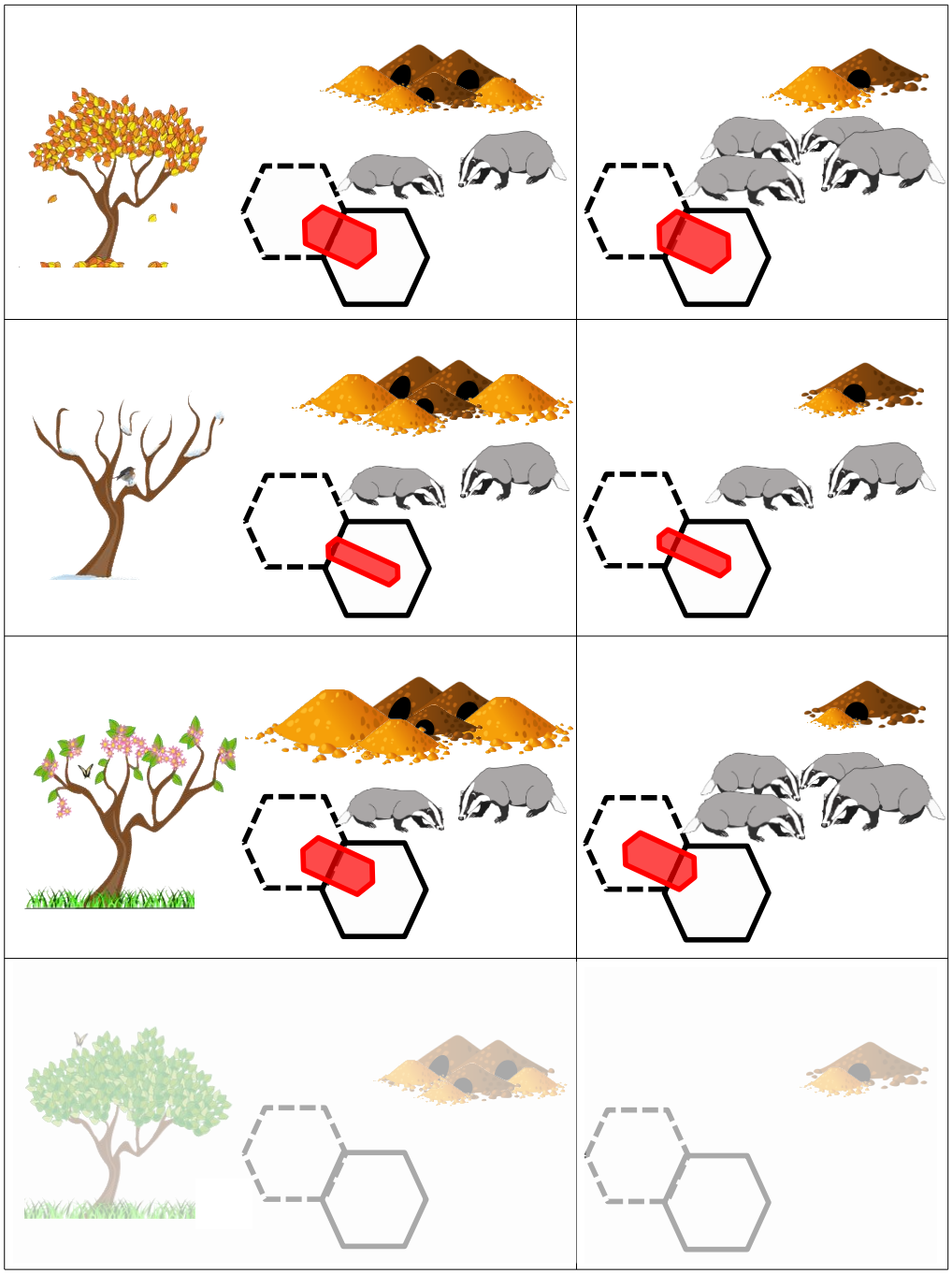


Figure 6.1 A calendar graphic of seasonal changes in badger sett use, ranging, and social behaviours. Behaviours associated with use of the social group's main sett (represented by multiple entrance holes and spoil heaps), and outlying setts away from the main sett (represented by a single entrance hole and spoil heap) are shown separately for each season. Spoil heap size increases with increasing frequency of sett use. Territories are depicted using black hexagons, with solid borders indicating the focal group territory, and dashed shapes indicating neighbouring group territories. Red polygons represent the badger home range size and extent of overlap with neighbouring territories. Red polygon widths increase with increasing range size, and extend

into home and neighbouring territories according to proportional territory-usage in each season. Badger icons represent the social position associated with home range overlap – network centrality increases with increasing number of badgers. Behaviours for each season are shown in the following order: autumn, winter, spring. Summer was not included in the analysis. For example, in *autumn* badgers use outlying setts more frequently: those badgers in outlying setts have similar range sizes to those at main setts, but are less likely to foray into neighbouring territories. They are subsequently more central in the badger social network.

6.3 Disease transmission

Community structure and disease transmission

When predicting disease spread through a population, mathematical models have traditionally assumed that all individuals share the same contact rates and probability of transmitting infection (Anderson *et al.* 1992; Begon *et al.* 2002; Keeling & Rohani 2008; Beldomenico & Begon 2010). These assumptions have little consequence for populations with homogenous contact structures (Keeling 2005; Bansal *et al.* 2007; Grassly & Fraser 2008). However, when applied to populations with variable contact rates, especially those with community structure, estimates lack accuracy and consistently underestimate disease spread (Keeling 2005; Bansal *et al.* 2007; Grassly & Fraser 2008; Jones & Salathe 2010). Therefore, the strong community structure identified in **chapter 2** confirms that using a modelling approach that accounts for variation in contact rates, such as network analysis, is necessary to study disease transmission in the Woodchester Park badger population.

Living in communities can be beneficial for individuals, for example by offering advantages when foraging and for predator defence (Krause & Ruxton 2002). However, group living can also be associated with elevated parasite burdens (Krause & Ruxton 2002), and increased probability of contracting infection from fellow group members (Jones & Salathe 2010), but when communities are isolated from the rest of the population the spread of disease can be inhibited. This is because the low density of connections between communities reduces the probability that disease will spread to another group (Liu *et al.* 2003; Wu & Liu 2008; Jones & Salathe 2010). This strong community structure can lead to

epidemics that are smaller than those in more homogenous populations (Wu & Liu 2008; Jones & Salathe 2010). Therefore, the strong community structure identified in **chapter 2** can explain why badger social structure restricts the spread of disease through the population (Delahay *et al.* 2000b), as limited contact between badger groups will reduce opportunities for transmission.

However, any contact that occurs between communities are likely to be highly important for disease spread; extra-group contact increases the probability that infection will spread between clusters, reducing the heterogeneity of the network and increasing the size of the epidemic (Wu & Liu 2008; Jones & Salathe 2010). Therefore, rigid separation of social groups may reflect secondary adaptations to reduce disease spread (Loehle 1995). Some species have developed ways to ensure communities remain isolated and prevent pathogens from entering. For example, some social insects exhibit social immunity to prevent infection from establishing within a colony. The benefits of this type of immune response extend beyond the individual (Cotter & Kilner 2010), and includes defences that rely on the cooperation of group members to control pathogens within the community (Cremer, Armitage & Schmid-Hempel 2007). For example, some insects guard nest entrances to prevent infected individuals from entering, and socially isolate infected individuals to prevent disease transmission (Cremer *et al.* 2007). However, motivation to increase foraging and breeding opportunities can override community isolation (Loehle 1995). For example lions (*Panthera leo*) reside in separate prides, but groups occasionally interact when prey is scarce (Packer, Scheel & Pusey 1990). In the Serengeti, these relatively rare between-pride contacts are sufficient to allow disease to spread across the whole population (Craft *et al.* 2011). Similarly, African buffalo (*Syncerus caffer*) reside in highly clustered herds, but drought results in increased herd mixing, increasing disease transmission through the population (Cross *et al.* 2004).

Extra-group contact between even the most isolated badger communities is therefore likely to be important for disease spread (**chapter 2, chapter 3, chapter 5**), and explains why an increase in badger movement between groups (Rogers *et al.* 1998; Vicente *et al.* 2007), and the breakdown of badger social structure in response to culling (Tuyttens *et al.* 2000; Woodroffe *et al.* 2006,

2008; Carter *et al.* 2007; Pope *et al.* 2007; Riordan *et al.* 2011; Bielby *et al.* 2014) can increase bTB incidence and prevalence rates. Further, extra-group contact was so common between some communities that they effectively functioned as a single group (**chapter 2**). This means that disease can potentially flow freely between these badger groups, possibly explaining why bTB infection is spatially aggregated within the population (Delahay *et al.* 2000b).

Furthermore, extra-group behaviour is highly seasonal, suggesting that among-group disease transmission may peak at certain times of year (**chapter 4, chapter 5**). The spring in particular is likely to represent a high-risk time for disease spread, with badgers that use outlier setts more likely to venture into neighbouring territories and hold network positions that increase the risk of acquiring infection *(*Christley *et al.* 2005; Figure 6.1). Conversely, in the autumn individuals with greater home range overlap are socially isolated in the population (**chapter 5**, Figure 6.1), suggesting that among-group disease transmission is likely to be low at this time. Peaks in disease transmission due to seasonal variation in contact rates can be observed in many species. For example, raccoons (*Procyon lotor*) spend more time together during the winter, leading to an increase in rabies transmission (Hirsch *et al.* 2016). Similarly, the Tasmanian devil (*Sarcophilus harrisii*) mating season is a key period for devil facial tumour disease (DFTD) transmission due to the associated increase in bite wounding (Hamede, Mccallum & Jones 2008).

The possibility that extra-group behaviour important for disease transmission is related to mating activity is a recurring theme in this thesis. In **chapter 3** I showed that extra-group contact was more likely to occur between less related individuals, possibly to avoid inbreeding. Many studies have shown experimentally that individuals prefer to mate with unrelated individuals (Pusey & Wolf 1996). For example, female three-spined sticklebacks (*Gasterosteus aculeatus*) will mate with unrelated males when given the choice (Frommen & Bakker 2006). Mating outside of the social group is one way to avoid inbreeding (Pusey & Wolf 1996). However, badgers are known to increase extra-group mating activity when within-group relatedness is high (Annavi *et al.* 2014), suggesting that individuals are able to recognise their kin.

The major histocompatibility complex (MHC) is a multigene family that influences the odour of the individual, making it an important cue used in kin recognition (Pusey & Wolf 1996). This olfactory cue has been shown to facilitate inbreeding avoidance in zebra fish (*Danio rerio*), where sexually mature females prefer the odour of unrelated males than related males (Gerlach & Lysiak 2006). In this case, mates are chosen to avoid inbreeding. The MHC is also important in controlling the vertebrate immune system (Klein 1986); MHC genes confer specific resistance to pathogens, and so individual fitness can be increased if heterozygosity at MHC loci is maximised (Penn, Damjanovich & Potts 2002). Therefore, mate choice can maximise offspring resistance to pathogens by selecting a mate with a greater MHC gene diversity. This type of mate choice has been observed in three-spined sticklebacks, where females prefer males with a higher diversity of MHC genes (Reusch *et al.* 2001). In this circumstance, pathogen resistance may be prioritised over inbreeding avoidance (Reusch *et al.* 2001).

Badgers are known to show variation in their MHC gene sequences (Sin *et al.* 2012). Therefore, given that the MHC gene complex influences *M. bovis* immunity in mice (Ladel, Daugelat & Kaufmann 1995), it may be possible that the MHC influences *M. bovis* susceptibility in badgers. MHC-based mate choice has been previously investigated in a badger population, with badgers found to select extra-group mates with similar MHC genes, possibly to reduce outbreeding of co-adapted gene complexes for local pathogens (Sin *et al.* 2015). However within-group mates were not selected in reference to MHC genes, and instead were selected to avoid inbreeding (Sin *et al.* 2015). This difference in mate choice could suggest that when mates are limited, inbreeding avoidance may override MHC optimisation (Sin *et al.* 2015). However, these findings are not echoed in my results; in **chapter 3** I showed that within-group contact was not associated with relatedness, but relatives spent less time together during among-group contact. Counter to the findings of Sin *et al*. 2015, my results suggest that inbreeding avoidance may drive extra-group mating. However, it is important to bear in mind that Sin *et al.* 2015 studied a different population of badgers that is not naturally infected with *M. bovis,* and so comparisons drawn between these studies should be treated with caution. In addition, **chapter 3** concerned all badger contact events, not just those related

to mating. Therefore, it is possible that contacts that occur during foraging or aggressive encounters may mask any correlations between relatedness and mate choice. Nevertheless, it would be interesting to determine if extra-group mating in the Woodchester Park badger population is motivated by inbreeding avoidance, or another driver such as MHC optimisation to confer resistance to TB.

If breeding behaviour motivates extra-group contact, this could suggest a trade-off between increasing individual fitness through breeding, and increasing the likelihood of acquiring infection. Disease transmission can be a cost of mating (Daly 1978), with some species empirically observed to have adapted to this cost through altering their breeding behaviour; DFTD is a highly infectious and virulent cancer of Tasmanian devils that peaks in transmission during the breeding season (Hamede *et al.* 2008). This disease has significantly reduced devil life expectancy, with individuals in infected populations compensating for this by changing their life history traits (Jones *et al.* 2008b); instead of older individuals breeding multiple times, devils are breeding only once and much younger (Jones *et al.* 2008b). In order for these changes in life history to be selected for, adult mortality has to be greater than that of juveniles (Charnov & Schaffer 1973). However, this is unlikely to be the case for less virulent diseases, such as bovine tuberculosis (Graham *et al.* 2013). Therefore, for populations infected with less virulent diseases, the trade-offs involved between mating success and disease acquisition are not likely to be as high, and so the occurrence of compensatory behaviours not as likely to occur.

Badger-badger disease transmission

My findings suggest that certain behavioural characteristics exhibited by some individuals may increase the risk of acquiring infection; in **chapters 4 and 5** I showed that home ranging behaviour explains more variation in network position and sett use compared to TB status. Previous studies have shown that TB test-positive badgers exhibit different behavioural characteristics compared to TB test-negative badgers. For example, TB test-positive individuals use outlier setts more frequently (Weber *et al.* 2013b), have larger home ranges (Cheeseman & Mallinson 1981; Garnett *et al.* 2005) and hold different network positions (Weber *et al.* 2013a). Given this evidence, it has previously been

suggested that TB infection may induce behavioural changes in badgers (Cheeseman & Mallinson 1981; Garnett *et al.* 2005). For example, infection can directly manipulate host behaviour by inducing phenotypic changes, as seen in rats (*Rattus norvegicus*) infected with *Toxoplasma gondii* (Berdoy *et al.* 2000). Alternatively, indirect behavioural changes can be observed, with infected individuals being less energetic as a product of their infection, as seen in sleepy lizards (*Tiliqua rugosa*) infected with ticks (Main & Bull 2000), or in the form of social exclusion, as seen in three-spined sticklebacks infected with ectoparasites (Dugatkin, Fitzgerald & Lavoie 1994). However, instead of TB causing these behavioural differences, my results suggest that behavioural differences may alter an individual's exposure to infection.

Environmental sources of infection are considered to be important in badger-cattle disease transmission (Drewe *et al.* 2013; Woodroffe *et al.* 2016). Therefore, it is possible that they are also important in badger-badger disease transmission. Previous studies have speculated that the sett might be an important environmental source of infection (Delahay *et al.* 2000b; Weber *et al.* 2013a), with the constant temperature, darkness and high levels of humidity creating an environment conducive to the survival of *M. bovis* (Roper 1992). *M. bovis* can be excreted in urine, sputum and faeces (Neal & Cheeseman 1996; Tuyttens *et al.* 2000; Delahay *et al.* 2000b), and so any excretions underground may result in a long-term source of infection. This would explain why TB test-positive individuals are found to use outlier setts more frequently (Weber *et al.* 2013a). However, in **chapter 4** I found that TB test-positive badgers were more likely to use outlier setts in the winter and spring, but not in the autumn. Therefore, if the sett were a dominant source of infection, this would fail to explain why badgers that used outlier setts in the autumn were not more likely to test positive for TB.

However, the role of environmental sources of *M. bovis* as focal points for disease transfer between badgers has not been widely considered and warrants further research. Along with the sett, badger latrines contain high concentrations of *M. bovis* (Hutchings & Harris 1999), posing a transmission risk to badgers that visit latrine sites. Similarly, badgers frequently forage in farm buildings where they contaminate and consume animal feed (Garnett,

Delahay & Roper 2002). There is potential to investigate these environmental sources of infection from a network perspective. A network approach has been used in previous studies, for example to determine the relationship between ectoparasite loads and crevice sharing in gidgee skinks (Godfrey *et al.* 2009); networks were built connecting lizards that subsequently used the same rock crevice, and found that infested lizards were more connected to other infested lizards (Godfrey *et al.* 2009). A similar approach could be taken to help determine if the sett, for example, is a source of infection for badgers; networks connecting badgers that subsequently use the same nest chamber could be built. If infected individuals are more connected, this could provide evidence for the sett being a source of infection. However, given that TB is predominantly transmitted via the respiratory route (Gallagher & Nelson 1979) and badgers often share nest chambers (Roper *et al.* 2001), differentiating between disease transmission as a product of resting location and social network position may be difficult.

In addition to indirect routes of disease transmission between badgers, direct transmission is also likely to play an important role. In **chapters 4 and 5** I showed that in the winter and spring, outlier sett use is associated with greater home range overlap, and consequently more central network positions. These network positions are known to reduce the protection group living can offer through increasing social group connectivity, and therefore increase the likelihood of acquiring infection (Altizer *et al.* 2003; Cross *et al.* 2004; Wu & Liu 2008; Jones & Salathe 2010). However, home range size was not related to social network position. This was unexpected given that an increase in range size in response to culling is thought to increase contact rates and, therefore, disease transmission (Woodroffe *et al.* 2006). Instead, my results suggest that home range overlap and the associated central network positions are likely to be more important for disease transmission than home range size. This may suggest that home range overlap might be more important during social perturbation events with regard to transmission risk (Woodroffe *et al*. 2006; Tuyttens *et al*. 2000; Riordan *et al*. 2011; Pope *et al*. 2007; Carter *et al*. 2007). Higher resolution spatial data, for example from GPS loggers, could help further determine the relationship between space use and disease transmission. However, GPS attachments to proximity collars are extremely expensive, and

may not provide the accuracy required given that badgers often spend time in valleys and woodland where GPS accuracy can be limited.

My findings suggest that some aspects of behaviour are likely to increase the risk of disease acquisition (**chapter 4, chapter 5**). However, these results should be interpreted with caution; exactly when individuals acquire TB can be hard to estimate, given that each badger is not necessarily caught at every capture event, and TB infection is easier to detect as infection progresses (Chambers *et al.* 2009). A longitudinal study is required in order to determine whether the behavioural differences observed in this research increases an individual's likelihood of acquiring infection, or if TB infection induces behavioural changes. Through following the same cohort of badgers, it could be determined if those that exhibit certain types of behaviour are more likely to acquire infection, or if badgers only exhibit these behaviours once they become infected. This would allow the relationship between badger behaviour and TB infection to be definitively determined.

In order to fully understand how a disease will spread through a population, variation within the social structure must be included in disease models (Lloyd-Smith *et al.* 2005). My research has shown that certain spatial behaviours are associated with social positions that will likely increase the probability of an individual acquiring infection (**chapter 5**). However, I have also shown that the relationships between these spatial and social behaviours change throughout the year (**chapter 4, chapter 5**, Figure 6.1). Therefore, although knowledge of a population's social structure can be essential when studying disease spread, this research has highlighted the additional importance of accounting for variations between these behaviours to fully understand the transmission of infectious diseases.

6.4 Disease management

Modelling studies have repeatedly shown that in populations where contact rates vary, targeting individuals that are important for disease transmission is more effective and efficient than random control (Liu *et al.* 2003; Lloyd-Smith *et al.* 2005; Krause *et al.* 2007; Grassly & Fraser 2008; Litvak-Hinenzon & Stone 2009; Jones & Salathe 2010). In some cases, a targeted approach can reduce

the amount of control required by up to 65% (Litvak-Hinenzon & Stone 2009). Therefore, given the clear heterogeneous contact structure that is present in this badger population (**chapter 2, chapter 3, chapter 5**), a targeted approach to bTB control is likely to be an effective one.

Potential control targets were identified in this research (**chapter 5**); badgers with greater home range overlap held more influential network positions for disease transmission (**chapter 5**, Figure 6.1). Therefore, if these individuals could be targeted with disease control, this could reduce disease transmission to the wider population. Similar strategies have been used to control disease in other species, such as Ethiopian wolves (*Canis simensis*) where individuals that bridge sub-populations were vaccinated to prevent further spread of rabies infection (Haydon *et al.* 2006). However, the seasonality in badger behaviour must also be considered. The behaviours that are likely to be important for disease transmission were only found in the spring (**chapter 5**, Figure 6.1), and therefore these individuals would need to be targeted before this time in order to prevent disease spread. Similar seasonal targeting of disease control has been suggested for raccoon populations, to prevent increased rabies transmission associated with den sharing in the winter months (Hirsch *et al.* 2016).

Although the efficacy of control strategies benefits from taking into account the social structure of the population (Liu *et al.* 2003; Lloyd-Smith *et al.* 2005; Grassly & Fraser 2008; Litvak-Hinenzon & Stone 2009; Jones & Salathe 2010), this information is not always available. However, individuals that are important for disease spread have been known to share distinguishing features. For example, deer mice (*Peromyscus maniculatus*) that are likely to be disproportionately responsible for the transmission of Sin Nombre virus are older and heavier (Clay *et al.* 2009). In **chapters 4 and 5** I found that individuals that used outlier setts in the spring were more likely to enter neighbouring territories, and subsequently hold influential network positions for disease spread (Figure 6.1). Therefore, outlier sett use in the spring could be used as a proxy to identify these high-risk individuals in other populations, with disease control targeted at these individuals early in the season to reduce disease transmission at this high-risk time.

High-risk individuals could be vaccinated with Bacillus Calmette-Guérin (BCG), which reduces the severity and progression of bTB (Chambers *et al.* 2011), and reduces the likelihood of an individual testing positive to diagnostic tests (Chambers *et al.* 2011; Carter *et al.* 2012). Badger vaccination trials have yielded promising results, with vaccinated individuals directly benefitting from the protective effects of BCG, and unvaccinated cubs indirectly benefitting from a reduced risk of acquiring infection (Carter *et al.* 2012). This indirect benefit to cubs is thought to be a product of herd immunity, where unvaccinated individuals are protected against disease transmission by the presence of vaccinated individuals (Topley & Wilson 1923). However, although the BCG vaccine reduces the severity of disease, it does not prevent an individual from becoming infected (Chambers *et al.* 2011). This type of vaccine has the potential to alter the selection pressures on pathogen virulence.

Pathogen virulence is a trade-off; it has to be high enough to prolong the infectious period and increase opportunities for disease transmission, but it cannot be so high that the host dies and the infectious period is reduced (Mackinnon, Gandon & Read 2008). This trade-off means an intermediate level of virulence is often optimal for pathogen transmission (Anderson & May 1982). However, if vaccination allows an individual to become infected but with a reduced risk of death, this means the pathogen is able to increase its virulence whilst avoiding the associated costs. Therefore, vaccination can lead to the evolution of hypervirulent pathogens, which puts unvaccinated individuals at greater risk from infection, instead of protecting them through herd immunity (Gandon, Mackinnon & Nee 2001). This type of pathogen evolution has been observed in malaria, where vaccination led to the evolution of a more virulent strain that was more damaging to unvaccinated hosts (Mackinnon & Read 2004).

It has been speculated that vaccinating human populations with BCG has led to the emergence of the hypervirulent Beijing strain of *M. tuberculosis*, although evidence is contradictory (Hanekom *et al.* 2011). However, the risk of a hypervirulent strain of *M. bovis* emerging in response to vaccination is likely to be low, given the low mutation rate and lack of genetic diversity of *M. bovis* in the UK (Smith *et al.* 2006), Nevertheless, consideration of the evolutionary

consequences of a targeted vaccination program to control bTB in badgers may be worth consideration, along with the practical aspects of deployment given that outlier setts can be numerous and hard to identify (Robertson *et al.* 2017; Roper 1992). Alternatively, individuals that use outlier setts in the spring could be selectively culled from the population, but given that even small scale removals are associated with perturbations (Bielby *et al.* 2014), the efficacy of this strategy would need to be trialled.

6.5 Conclusions

It is clear that the community structure, and social structure generally, of a population can influence the spread of disease. However, some traditional models of disease transmission do not acknowledge this variation, instead assuming all individuals to have the same number of contacts based on the population average (Anderson *et al.* 1992; Begon *et al.* 2002; Keeling & Rohani 2008; Beldomenico & Begon 2010). The accuracy of these models is, therefore, compromised when applied to populations with heterogeneous contact structures, especially those with community structure (Keeling & Eames 2005; Bansal *et al.* 2007; Grassly & Fraser 2008; Jones & Salathe 2010). My research has used social network analysis to investigate the community structure and space use of a social mammal, to explore the implications for disease transmission. Through using empirical data to build weighted networks, contacts that are likely to carry a transmission risk were analysed to identify high-risk individuals, and behaviours, that are likely to be important for disease spread. The social structure identified suggests that the community structure is likely to be highly influential in mitigating, but also facilitating, the spread of disease through the population. Social network analysis is clearly a highly valuable tool to allow heterogeneous contact structures to be accounted for when studying the spread of disease, where the use of traditional models may not be appropriate.

Knowledge of the population social structure can be exploited to design targeted disease control strategies, which are likely to be more effective than blanket policies for populations that show such heterogeneities in their contact structures. The need to effectively control wildlife diseases is clear; they pose a significant threat to species of conservation concern, where infection can lead

to further declines in already endangered species (e.g. Woodroffe & Ginsberg 1999; McCallum *et al.* 2009). In addition, wildlife diseases cause great economic loss to agriculture through the infection of livestock (e.g. Michel *et al.* 2009; Seleem *et al.* 2010). However, perhaps the greatest pressure to understand and control wildlife disease is the public health risk that they pose; zoonotic diseases make up approximately 70% of human emerging infectious diseases (Taylor *et al.* 2001; Jones *et al.* 2008a), with mammals representing a particularly important source of emerging disease (Cleaveland, Laurenson & Taylor 2001). These emerging diseases from wildlife sources represent a significant and growing risk to public health (Jones *et al.* 2008a). However, the mechanisms that facilitate the transition of infections from wildlife to persisting in humans remain unclear (Perkins, Cattadori & Hudson 2005). Understanding disease spread through wildlife populations can help give insight into parts of this process, allowing risks to be managed and effective control strategies to be planned. This thesis has demonstrated the substantial role social network analysis can play to allow this understanding to be achieved.

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“One good idea could cost you thousands of your days, but it’s just time you would be spending anyway”

Jeffrey Lewis, Time Trades